

Co-cultivation of mast cells and FcεRIα⁺ dendritic-like cells from human hip bone marrow

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Summary

Background Mast cells contribute to the pathogenesis of asthma and allergy through the release of a plethora of pro-inflammatory mediators and cytokines. Their study is hampered by the difficult access to human tissue samples. Human mast cells have been cultured from CD34⁺ progenitors in the bone marrow of normal volunteers following iliac crest bone marrow biopsy but this is invasive. Hip bone marrow could provide a more convenient less invasive source of mast cell progenitors.

Objective To characterize mast cells cultured from human bone marrow obtained at routine hip surgery.

Methods Mononuclear cells were isolated from the bone marrow reamings of patients undergoing routine hip replacement surgery and were cultured with recombinant stem cell factor (SCF), IL-6 and IL-10. Cell surface markers were examined using flow cytometry, protease expression monitored using immunohistochemistry, histamine measured by radioenzymic assay, Ca²⁺ responses analysed using ratiometric Ca²⁺ imaging, and ion currents recorded via the patch-clamp technique.

Results Mast cells were absent at baseline, but accounted for 65 ± 7% of cells after 8–12 weeks of culture, equating to a mean 0.6 ± 0.14 × 10⁶ mast cells per culture. Fifty-three percent of tryptase⁺ cells also contained chymase. The remaining cells comprised a population of large CD1a⁺ HLA-DR⁺ and FcεRIα⁺ cells, most likely dendritic cells. All mast cells expressed CD117 and the high-affinity IgE receptor α-chain (FcεRIα) constitutively, and developed a Ca²⁺ response following IgE-dependent activation. These cells exhibited 7.8 ± 2.9% net IgE-dependent histamine release, and demonstrated a similar ion channel profile to human lung mast cells. In particular, the intermediate conductance Ca²⁺-activated K⁺ channel opened following IgE-dependent activation.

Conclusions Mast cells grown from human hip marrow provide a rich non-invasive source of functionally mature mast cells. In addition, this culture system may be useful for the generation of FcεRIα⁺ dendritic cells.

Keywords bone marrow, Ca²⁺, CD1a, dendritic cell, FcεRIα, human, ion channel, mast cell

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Introduction

Mast cells play a pivotal role in the pathophysiology of asthma, allergic rhinoconjunctivitis and anaphylaxis through their immunomediator-secretory activities in response to activation by both allergen and other secretory stimuli [1]. Histamine, PGD₂ and LTC₄ contribute to mucosal symptoms through the induction of mucus secretion and mucosal oedema, and in the case of asthma, bronchoconstriction. Mast cells also synthesize and secrete a number of pro-inflammatory cytokines including IL-4, IL-5 and IL-13, which regulate both IgE synthesis and the development of eosinophilic inflammation. In addition, the mast cell proteases tryptase and chymase have the potential to cause tissue damage as well as contributing to the inflammatory response.

Importantly, mast cells are present in an 'activated' secretory state in these diseases, with evidence of ongoing mediator release, degranulation visible with electron microscopy, and cytokine mRNA synthesis [1].

Mast cells are derived from progenitor cells in the bone marrow, which circulate as undifferentiated CD34⁺ mononuclear cells in the peripheral circulation [2]. It is following migration into tissue that maturation occurs, presumably under local influences. Stem cell factor (SCF) is the critical growth and differentiation factor for human mast cells, and is an essential ingredient for the culture of human mast cells from immature precursors in bone marrow, umbilical cord blood, peripheral blood and fetal liver [2]. The role of other cytokines is less clear, although IL-6 seems to potentiate mast cell growth in the presence of stem cell factor [3].

The study of human mast cells is of great interest for understanding the pathogenesis of allergic disease, but is hampered by the difficulty in obtaining satisfactory human tissue samples for cell isolation, and the relative paucity of

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cells obtained. A tissue source of interest is human bone marrow, but the methods for the culture of mast cells from human bone marrow described previously have utilized marrow obtained from normal donor iliac crest. This is relatively invasive and the donor cell preparation has involved the purification of $CD34^+$ progenitors prior to culture [4]. The principal aim of this study was to investigate the suitability of utilizing adult hip bone marrow obtained from routine surgery for the culture of human mast cells. The human bone marrow mast cells (HBMMC) cultured have been characterized with respect to surface receptor and protease expression, histamine secretion, Ca^{2+} responses and ion channel physiology.

Material and methods

Reagents and cytokines

Dulbecco's modified eagle's medium with HEPES and glutamax (DMEM), non-essential amino acids solution, heat-inactivated fetal bovine serum (FBS), and Hanks' balanced salt solution without Ca^{2+} and Mg^{2+} (HBSS) were purchased from Invitrogen Life Technologies (Paisley, Scotland, UK). Antibiotic/antimycotic solution, cell dissociation solution (non-enzymatic), bovine serum albumin (BSA), goat polyclonal anti-IgE, histopaque-1077, paraformaldehyde, Tween 20, trypan blue, fura-2-acetoxymethyl ester (Fura-2-AM), 4',6-diamidino-2-phenylindole (DAPI), clotrimazole and 1-ethyl-2-benzimidazolinone (1-EBIO) were all purchased from Sigma (Poole, Dorset, UK). Human myeloma IgE was purchased from Calbiochem-Novabiochem (Nottingham, UK). Histamine and *s*-adenosyl-L-methionine- ^3H methionine were purchased from Amersham Life Science (Little Chalfont, Bucks, UK); rat kidney histamine methyl transferase was a generous gift from Dr S. Harper (Astra Charnwood, Loughborough, UK). SCF, IL-6 and IL-10 were obtained from R&D Systems (Abingdon, Oxfordshire, UK).

Antibodies

The following mouse IgG mAbs were purchased from DAKO (Glostrup, Denmark): the antigens they are directed against are shown in brackets, i.e. clone (antigen). PE-conjugated YB5.B8 (CD117), FITC-conjugated NA1/34 (CD1a), and unconjugated TUK4 (CD14), CR3/43 (HLA-DR), DJ130c, (CD16) and AA1 (tryptase). The CD83 mAb, HB15e was purchased from Caltag Laboratories (Burlingame, CA, USA). The FITC-conjugated W6/32 (HLA-class I) mAb was purchased from Sigma. The biotinylated AA5 (tryptase) mAb was purchased from Promega, Madison, WI, USA) and the MAB1254 (chymase) mAb from Chemicon (Temecula, CA, USA). The rabbit anti-human $Fc\epsilon RI\alpha$ polyclonal antibody (rabbit antiserum, Cat No: 06-725) and the mouse anti-human mAb 3G6 were purchased from Upstate Biotechnology (Lake Placid, New York, USA).

Cell culture

Human bone marrow was obtained from patients undergoing routine hip replacement surgery. The patients gave written informed consent and the study was approved by the Leicestershire Ethics Committee.

Reamings from the femoral shaft were collected in heparinized tubes in DMEM supplemented with 10% (v/v) FBS, 1% antibiotic antimycotic, 1% non-essential amino acids. The heparinized sample was diluted 1:1 with HBSS with 0.5% FBS. The cell suspension was carefully layered above histopaque-1077 and centrifuged at 400g for 30 min at room temp. The cell layer at the interface was transferred into a fresh tube and diluted 1:4 with HBSS before being centrifuged at 400g. The cells were washed twice in HBSS. Finally, the cells were re-suspended in 1 mL of DMEM and counted in a Coulter Ac.T cell counter (Coulter Corporation, Miami, FL, USA).

The cell suspension was cultured with SCF (100 ng/mL), IL-6 (50 ng/mL) and IL-10 (10 ng/mL), respectively, for up to 12 weeks at 37 °C in a 5% CO_2 humidified atmosphere. Half the medium was replaced with fresh medium every 7 days with the same concentration of SCF, IL-6, and IL-10. A proportion of the suspended cells were stained with Kimura to determine the metachromatic cells at each time point. Cell viability was determined with trypan blue. More than 95% of the cells were viable at each time point.

Flow cytometry

For cell surface receptor expression, $0.8 - 1 \times 10^5$ cells/tube were re-suspended in phosphate-buffered saline (PBS), 0.5% BSA. Non-specific antibody binding was blocked using mouse IgG. The cells were then incubated with a directly conjugated mAb against CD117-RPE or antibodies to CD1a, CD14, CD16, HLA-class I and $Fc\epsilon RI\alpha$, which were indirectly labelled with FITC-labelled secondary antibodies where necessary. Appropriate isotype controls were performed.

Immunohistochemistry

Cytospins were prepared from the cell suspension, fixed with paraformaldehyde for 20 min, washed, and then blocked with 0.1% BSA and 20% FCS for 30 min. They were first incubated with an appropriate antibody overnight, and then biotinylated streptavidin peroxidase. 3-Amino-ethylcarbazole was added as the chromogen producing a red reaction product. The cells were counterstained with haematoxylin.

Immunofluorescence

The cell suspension was cultured on fibronectin-coated eight well chamber slides for 3 days. Cells were then fixed with ice-cold methanol for 20 min, blocked with PBS/3% BSA for 30 min at room temp., and then incubated with the anti-chymase antibodies for 90 min. After washing $3 \times$ with PBS/0.05% Tween 20, a secondary FITC-labelled antibody was added for 90 min. After rinsing $6 \times$, a biotinylated tryptase antibody was added for 90 min, rinsed with PBS/Tween 20, and finally streptavidin Texas red was added for 90 min. The slide was then rinsed $3 \times$ and stained with 0.2 μ g DAPI. The slide was then mounted with fluorescent mounting medium and analysed.

Calcium imaging

Changes in cytosolic-free Ca^{2+} concentration ($[Ca^{2+}]_i$) were monitored fluorometrically by use of the Ca^{2+} -sensitive probe

Fura-2. Briefly, cells were first sensitized with human myeloma IgE 2.5 µg/mL for 30 min, washed and then loaded with Fura-2 by incubation in normal physiological saline solution (PSS: 130 mM NaCl, 5.6 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 11 mM glucose, 10 mM HEPES, pH 7.4 with NaOH) containing 2 µM Fura-2-AM for 30 min at room temperature and then washed in PSS for 30 min. A cover slip with attached cells was mounted in a perfusion chamber at 37 °C. The recording system included a Nikon TE200 inverted microscope fitted with epifluorescence (Nikon, UK, Kingston-upon-Thames, UK) equipped with a $\times 40$ Plan Fluor objective (Nikon, UK). Light from a mercury arc lamp (Nikon, UK) was passed alternately through excitation band-pass filters of 340 and 380 nm, which were exchanged by a computer-controlled filter wheel unit (Ludl Electronic Products, Hawthorne, NY, USA). A 430-nm long-pass dichroic mirror and 510-nm long-pass emission filter supplied light to the Hamamatsu CCD camera (Hamamatsu Photonics, Hamamatsu City, Japan). All optical filters were from Omega Optical (Brattleboro, VT, USA). Data were collected using Openlab version 3.1 software (Improvision, Coventry, UK). Data acquisition occurred at a rate of one dual-wavelength image every 6 s as the 340/380 nm ratio. This was converted to $[Ca^{2+}]_i$ using a commercially available calibration kit (Molecular Probes, Eugene, OR, USA). Drugs were added to the dish as required.

Electrophysiology

The whole-cell variant of the patch-clamp technique was used [5]. Patch pipettes were made from boro-silicate fibre-containing glass (Clark Electromedical Instruments, Reading, UK), and heat polished resulting in resistance of 4–6 MΩ. The standard pipette solution contained (mM) KCl, 140; MgCl₂, 2; HEPES, 10; Na⁺-ATP 2, GTP 0.1, pH 7.3. The standard external solution contained (mM) NaCl, 140; KCl, 5; CaCl₂, 2; MgCl₂, 1; HEPES, 10, pH 7.3. For recording, mast cells were placed in 35 mm dishes containing standard external solution.

Whole-cell currents were recorded using an Axoclamp 200A amplifier (Axon Instruments, Foster City, CA, USA), and currents usually evoked by applying voltage commands to a range of potentials in 10 mV steps from a holding potential of -20 mV. The currents were digitized (sampled at a frequency of 10 kHz), stored on computer and subsequently analysed using pClamp software (Axon Instruments).

Capacitance transients were minimized using the capacitance neutralization circuits on the amplifier. Correction for series resistance was not routinely applied. Experiments were performed at 27 °C, temperature being controlled by a Peltier device, and a perfusion system (Automate Scientific Inc., San Francisco, CA, USA) was used to allow solution changes. Drugs were added directly to the recording chamber.

Mast cell activation

For analysis of histamine release, 1×10^4 mast cells were warmed to 37 °C in triplicate in 50 µL of DMEM, and 50 µL of medium containing $2 \times$ the final concentration of goat polyclonal anti-human IgE (1:2000 final dilution, which gives maximal histamine release from human lung mast cells (HLMC)) was then added. After 30 min incubation at 37 °C, the cells were centrifuged at 250 *g* for 4 min, the supernatant decanted, and control cell pellets lysed in sterile de-ionized water for measurement of total histamine content as described previously [6].

Data analysis

Histamine release was expressed as net% release of total cell content or nanogram per 10^6 cells as appropriate. Differences between experimental conditions were explored using Student's *t*-test, and *P*-value <0.05 accepted as statistically significant.

Results

Isolation and differentiation of bone marrow mononuclear cells

A mean $13.3 \pm 1.8 \times 10^6$ mononuclear cells were isolated from surgically obtained hip bone marrow, of which $53.7 \pm 2.64\%$ were lymphocytes, $9.7 \pm 1.2\%$ monocytes and $36.6 \pm 2.2\%$ contaminating granulocytes. These cells, which contain haemopoietic progenitor cells, were cultured in the presence of SCF, IL-6 and IL-10 for up to 12 weeks. Mast cells, determined by metachromatic staining, were absent at baseline, but accounted for $65 \pm 7.2\%$ of cells after 8–12 weeks of culture, equating to a mean $0.6 \pm 0.14 \times 10^6$ mast cells per culture. Interestingly, a second population of morphologically distinct cells developed. These were strongly adherent cells with a dendritic morphology, and when

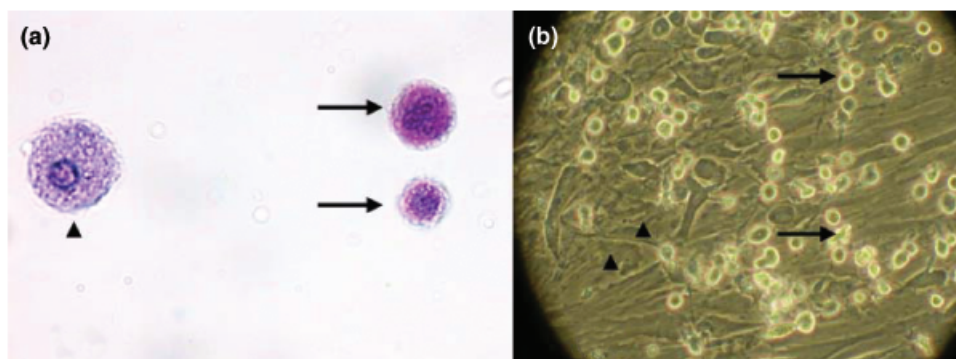


Fig. 1. (a) Kimura stain of detached cells demonstrating two distinct populations of small granular mast cells (arrows) and large vacuolated cells (arrowheads). (b) Phase contrast image of adherent cells in tissue culture demonstrating dendritic-like cell morphology (arrowheads) with some mast cells (arrows) adhered to them.

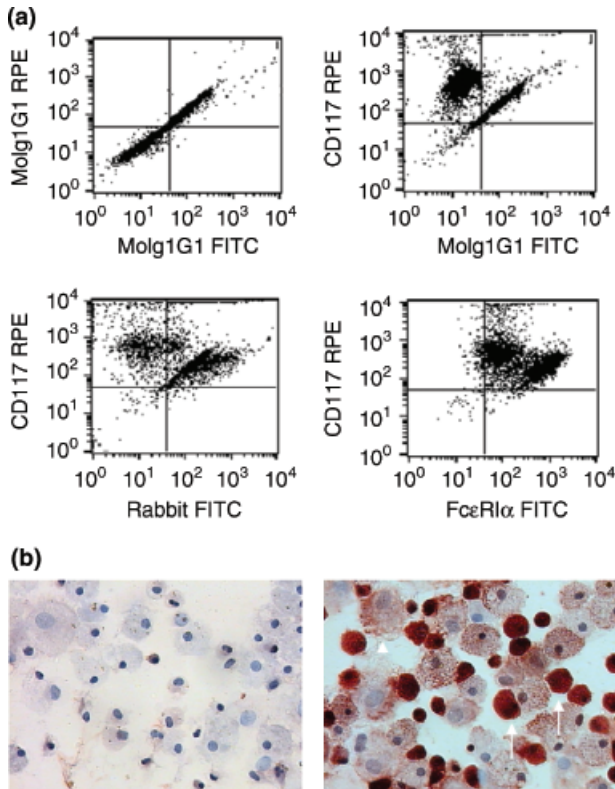


Fig. 2. (a) Representative example of flow cytometric analysis of human bone marrow mast cells (HBMMC) surface CD117 and $Fc\epsilon RI\alpha$ expression. (b) Immunohistochemistry for tryptase demonstrating strong tryptase immunoreactivity within mast cells (arrows), and occasional weak staining in the dendritic-like cell population (right panel). Isotype control staining is shown in the left panel.

detached were noted to be large cells with small round nuclei (Figs 1a and b). These cells accounted for $30 \pm 5.9\%$ of cells present at 12 weeks. A subpopulation of the mast cells were adherent to the dendritic-like cells.

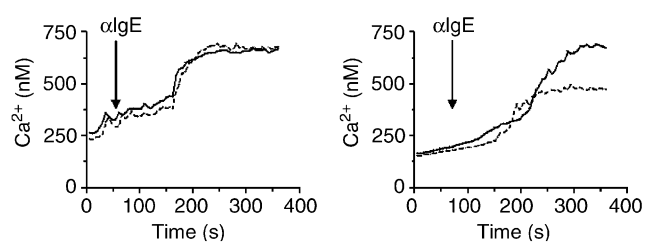


Fig. 3. Representative changes in cytosolic-free Ca^{2+} concentrations ($[Ca^{2+}]_i$). Each panel is from a separate donor and each trace represents an individual human bone marrow mast cell activated with anti-IgE at the times indicated.

Characterization of human bone marrow mast cells

The surface expression of antigens on HBMMC was investigated by flow cytometry (Fig. 2a). Of note, both CD117 and the α chain of the high-affinity IgE receptor ($Fc\epsilon RI\alpha$) were expressed constitutively on all the mast cells. The protease content of HBMMC was detected using standard immunohistochemistry where the cells were strongly positive for tryptase (Fig. 2b). HBMMC contained 1.9 ± 0.11 pg histamine per cell and exhibited $7.8 \pm 2.9\%$ net histamine release following anti-IgE-dependent activation with a 1:2000 dilution of anti-IgE.

Ratiometric Ca^{2+} imaging on single Fura-2-loaded HBMMC demonstrated that 100% of the mast cells developed a rise in $[Ca^{2+}]_i$ following IgE-dependent activation with a net increase of 364.7 ± 22.4 nM Ca^{2+} ($P < 0.0001$, three donors, 15 cells, Fig. 3).

Whole-cell patch-clamp recording demonstrated a similar ion channel profile to HLMC. Thus, at rest, there was marked heterogeneity in the currents present so that out of 30 cells recorded, 10 cells were electrically silent, 12 cells expressed small outwardly rectifying currents with reversal potential around 0 mV but which rapidly ran down, four cells expressed small linear currents with reversal potential around 0 mV,

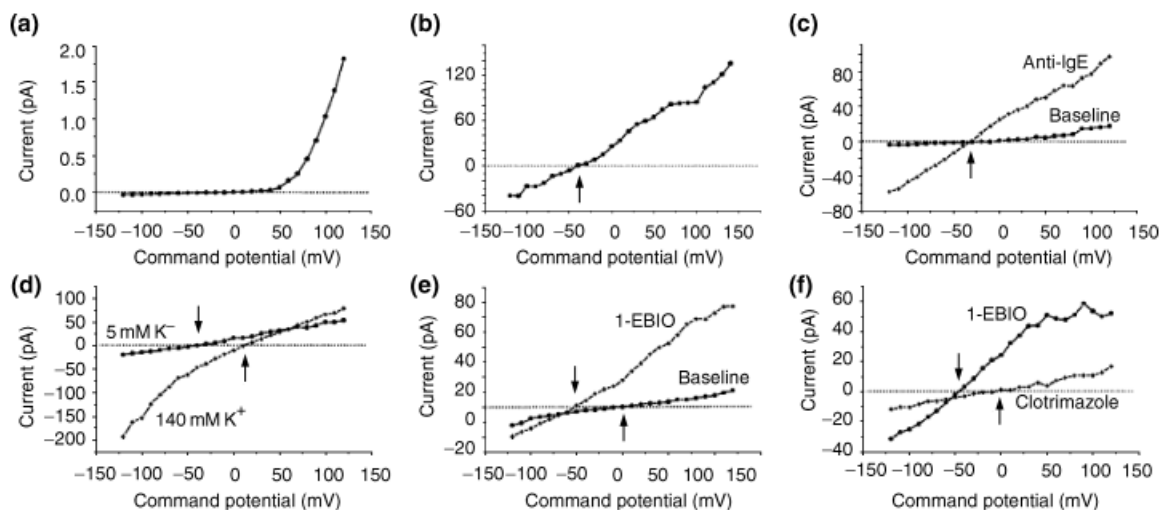


Fig. 4. Whole-cell electrophysiological recording of human bone marrow mast cells demonstrating electrical currents present at rest and following IgE-dependent activation. (a) Current-voltage (I - V) curve of a CIC-5-like current in a cell at rest. (b) I - V curve of a dominant iK_{CA1} current in a cell at rest. Note the negative reversal potential (arrow). (c) I - V curves of a cell expressing minimal current at rest, and then 2 min after IgE-dependent activation demonstrating the development of a dominant iK_{CA1} current with an associated negative shift in reversal potential (arrow). (d) I - V curves of a cell demonstrating the iK_{CA1} current after IgE-dependent activation recorded in 5 and 140 mM external K^+ . Note the positive shift in reversal potential (arrows) in 140 mM K^+ . (e) I - V curves of a cell at rest and following exposure to the iK_{CA1} opener 1-EBIO. (f) I - V curves of a cell demonstrating the iK_{CA1} current after exposure to 1-EBIO and its block by clotrimazole $1 \mu M$. Note the positive shift in reversal potential following K^+ channel blockade (arrows).

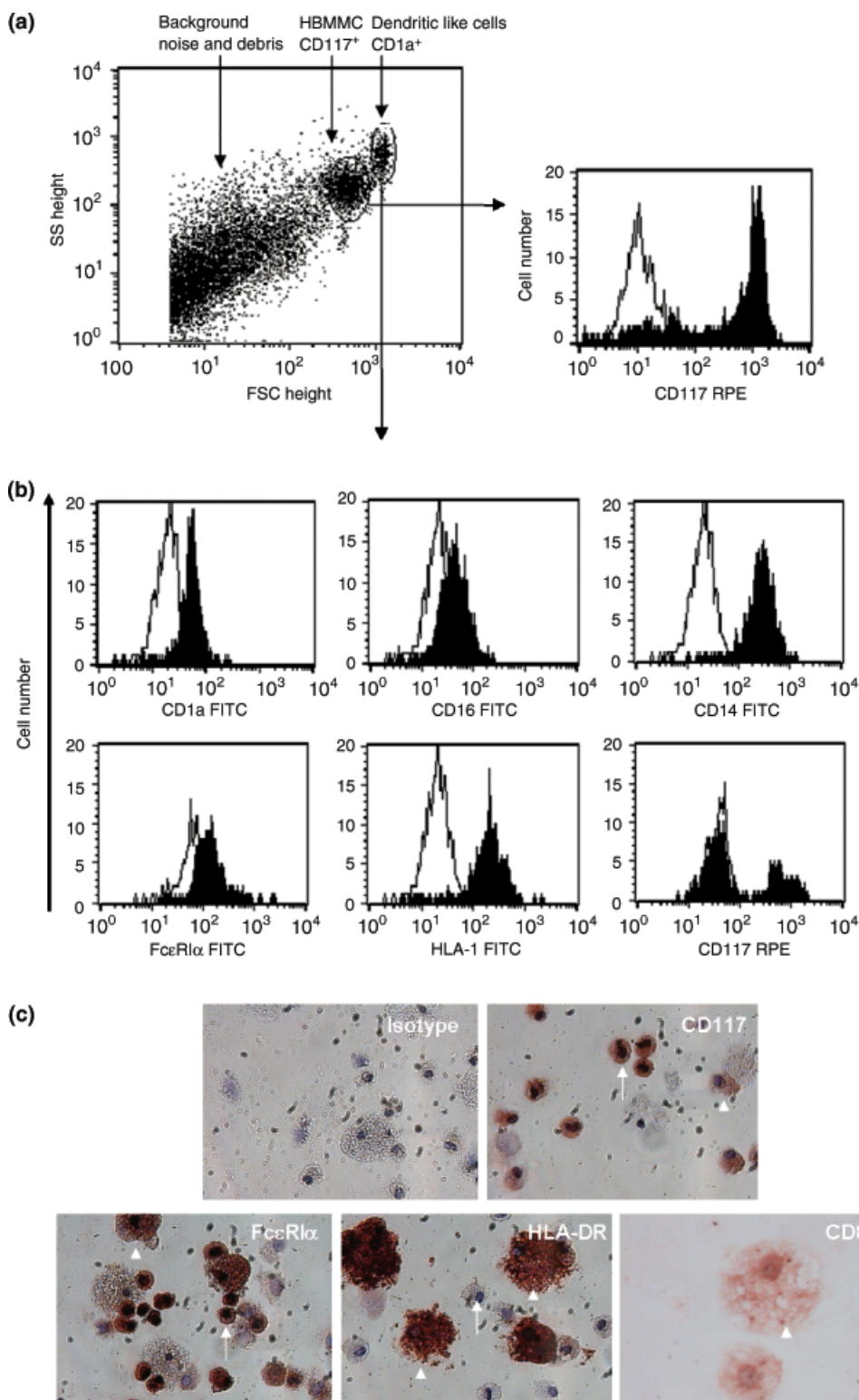


Fig. 5. Analysis of cells after 12 weeks of culture using forward scatter and side scatter parameters demonstrating two populations of cells. (a) One population of single human bone marrow mast cells (HBMMC) positive for CD117 and the other (b) larger dendritic-like cell population positive for CD1a, CD16, CD14, FcεRIα and HLA-1. A sub-population of HBMMC adherent to the larger cells were positive for CD117 as demonstrated in the fluorescent histograms. (c) Immunohistochemistry staining with CD117 in single HBMMC (arrows) and HBMMC adherent to larger cells (arrowheads). FcεRIα staining was intense within the HBMMC (arrows) and weak to strong within the larger cells (arrowheads). HLA-DR staining was intense within the larger cells (arrowheads) and there was no staining in HBMMC (arrows). CD83 staining was detected in the dendritic-like cells. The isotype control is demonstrated in the top left panel.

two cells expressed the CIC-5-like outwardly rectifying Cl^- current and two cells expressed the K^+ current carried by intermediate conductance Ca^{2+} -activated K^+ channel (iK_{CA1}) (Figs 4a and b). Following IgE-dependent activation, iK_{CA1} opened in 5/11 cells resulting in membrane hyper-

polarization (Fig. 4c). Switching the external recording solution to one containing 140 mM K^+ produced an appropriate positive shift in reversal confirming that this current was carried by K^+ (Fig. 4d). This current was also opened by the iK_{CA1} opener 1-EBIO in 5/11 cells (Fig. 4e),

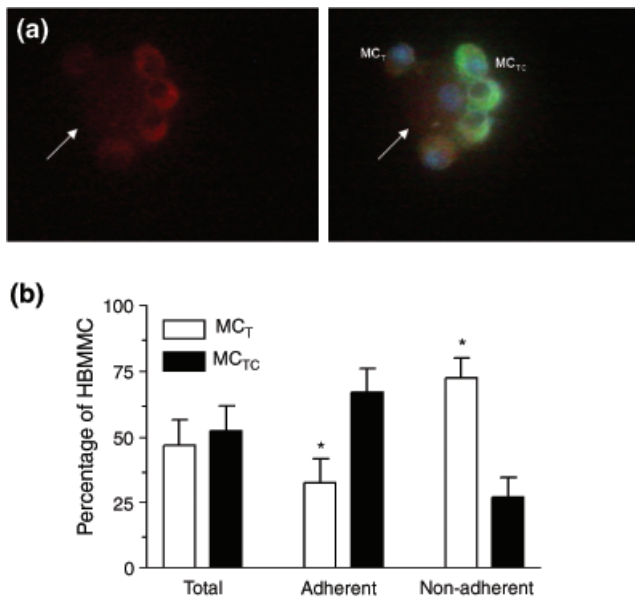


Fig. 6. (a) Immunofluorescence demonstrating the adherence of human bone marrow mast cells (HBMMC) to the dendritic-like cells. Tryptase-only (MC_T) mast cells clustered around a dendritic-like cell (arrow). The right-hand panel demonstrates the same cells stained with triple fluorescence showing adherence of chymase plus tryptase positive (MC_{TC}) HBMMC (green). Nuclei stained blue with DAPI. (b) Bar graph of mean (SEM) of the proportion of HBMMC adherent to the dendritic-like cell population compared to non-adherent cells (three donors, * $P < 0.05$).

and blocked by the iK_{CA}1 blocker clotrimazole in 5/5 cells (Fig. 4f).

Characterization of the dendritic-like cell population

Cultured cells were analysed by flow cytometry for surface antigen expression. Figure 5a shows the size and granularity of the cells and clearly two distinct populations were identified. All cells were analysed for CD1a, CD16, CD14, HLA-1 and CD117. When the cells were back-gated into the initial dot plot graph, cells that were positive for CD1a, CD16 and CD14 clearly lay within the large cell population, and the cells that were highly positive for CD117 were largely located within the smaller subpopulation (Figs 5a and b). However, a subpopulation of cells within the larger population were also CD117 positive ($13.6 \pm 7.8\%$), which were likely to represent adherence between dendritic-like cells and CD117 HBMMC. Of great interest, the dendritic-like cells were also positive for the α chain of FcεRI (Fig. 5b). The FcεRIα surface expression was independent of HBMMC as only a small proportion of cells were positive for CD117 within the large population of cells, whereas $42.3 \pm 12.7\%$ of the large dendritic-like cells shifted into the FcεRIα-positive region of the flow cytometric histogram (Fig. 5b). Furthermore, the dendritic-like cells stained for FcεRIα using immunohistochemistry (Fig. 5c), and the FcεRIα on these cells appeared to be functionally active in that activation with anti-IgE induced a Ca²⁺ response (baseline [Ca²⁺]_i 385 ± 182 , peak Ca²⁺ post-anti-IgE 632 ± 190.5) in two cells. Lastly, the large dendritic-like cells also stained for HLA-DR and CD83 using immunohistochemistry (Fig. 5c), which when taken together with the expression of CD1a evident in the FACS, strongly suggests that they are of dendritic origin.

Potential interaction of dendritic-like cells and human bone marrow mast cells

We noted in tissue culture conditions that a subpopulation of HBMMC were adherent to the dendritic-like cells (Fig. 1b), which might be important in determining the phenotype of each cell type. Firstly, the dendritic-like cells were slightly positive for tryptase when compared with control cells suggesting they may have phagocytosed mast cell granules/endosomes (Fig. 2b). When looking at the mast cell phenotype, the overall percentage of HBMMC that were tryptase-only positive (MC_T) was $47.3 \pm 9.6\%$ and chymase plus tryptase positive (MC_{TC}) $52.7 \pm 9.6\%$. Interestingly, MC_{TC} accounted for $67.2 \pm 9.2\%$ of the mast cells adherent to the dendritic-like cells and only 27.3 ± 7.7 of the non-adherent mast cells ($P < 0.05$, Fig. 6). These observations raise the possibility that the dendritic-like cells may play an important role in the maturation of the MC_{TC} HBMMC phenotype, and conversely, the MC_{TC} mast cells may contribute to the maturation of FcεRIα⁺ dendritic cells.

Discussion

We have shown that it is possible to grow functionally mature mast cells from human hip bone marrow obtained from routine hip surgery. The yield of mast cells was similar to that described by Shimizu et al. [4], who obtained bone marrow from the iliac crest of normal volunteers. However, in view of the non-invasive nature by which we obtained marrow, and the widespread access to routine hip surgery, we suggest that our method is particularly suitable for the study of HBMMC.

In this study, we used the whole bone marrow mononuclear preparation rather than isolated CD34⁺ progenitors as described recently by others [4]. The characteristics of the mast cells we generated were broadly similar to this recent report but there were also some notable differences. In keeping with the report by Shimizu and colleagues, our cells constitutively expressed high levels of FcεRIα and CD117, and contained a similar amount of histamine (approximately 2 pg/cell). We found that about 50% of our HBMMC cells expressed chymase, which is relatively high compared with suspension cultures from other progenitor sources such as cord blood, but lower than the 100% reported by Shimizu et al. [4]. Since the origin of MC_T vs. MC_{TC} cells is unclear, this observation may relate to the fact that we have used the whole bone marrow preparation rather than CD34⁺ progenitors. In addition, our culture conditions were slightly different in that we also used IL-10 to suppress monocyte development [7]. However, it is unlikely that this explains the decreased expression of chymase since IL-10 is reported to increase expression of two rodent mast cell chymases [8]. A further possibility is that the contaminating CD1a⁺ cells in our cultures suppress chymase expression, but this seems unlikely because HBMMC adherent to these cells were actually enriched for chymase expression.

By 12 weeks, our HBMMC did not reach the >95% purity described previously [4]. This was because of the co-cultivation of a second population of morphologically distinct CD1a⁺ HLA-DR⁺ cells. These cells were readily identifiable compared with the HBMMC because of their dendritic appearance when adherent, and their relatively large size and

small round nucleus, which was evident when they were detached. Similar cells have been observed in some previous HBMMC cultures but assumed to be macrophages [9, 10]. However, the expression of CD1a, CD83 and HLA-DR together with their adherent morphology suggests they are likely to be of dendritic origin [11–14]. This is perhaps not surprising as dendritic cells are relatively easy to culture from CD34⁺ cells in a variety of cytokines including SCF and IL-6. The dendritic-like cells in our cultures are of great interest because they expressed FcεRIα, which appeared functional judging by the rapid increase in [Ca²⁺]_i following FcεRIα cross-linking with anti-IgE. The factors leading to the differentiation of dendritic cells expressing surface FcεRIα is poorly defined, but IL-4 increases the intracellular expression of FcεRIα, although not the surface expression [9]. Since the HBMMC in this study were clearly adherent to the dendritic-like cells, we can speculate that HBMMC-derived factors might contribute to dendritic cell FcεRIα expression.

In this study, we have also examined the Ca²⁺ response and ion channel profile of HBMMC. The Ca²⁺ response following IgE-dependent activation was broadly similar to that described previously in HLMC [15, 16] with a typical spike and plateau representative of Ca²⁺ release from internal stores and influx from the extracellular milieu, respectively. In addition, the plateau was relatively smooth in that the wide Ca²⁺ oscillations typical of basophil activation were absent [15]. We have recently described a tight correlation between the change in [Ca²⁺]_i and net histamine release following IgE-dependent activation in HLMC [17]. However, the HBMMC generated in this study do not fit this relationship. Thus, while our HBMMC exhibit a large increase in [Ca²⁺]_i, histamine release was relatively poor, suggesting that part of the FcεRIα signalling pathway or the secretory apparatus in these cells is not fully developed.

An emerging area of interest in human mast cell biology is the regulation of cell function by ion channels [17–21]. Ca²⁺ influx is essential for many cell processes, and K⁺ and Cl[−] channels have important regulatory roles on Ca²⁺ signals through their effects on cell membrane potential. Thus, K⁺ and Cl[−] currents also have important roles in cell responses such as secretion, proliferation and chemotaxis [19, 22]. The ion currents present in HBMMC were broadly similar to those seen previously in human lung and peripheral blood-derived mast cells [19], and clearly distinct from those described in rodents. This indicates that HBMMC are a suitable and convenient model for the study of human mast cell ion channel physiology. For many studies, it is not essential to have highly purified cell populations, but where this is necessary, it is straightforward to positively select the cells of interest using immunomagnetic affinity selection as described previously for cultures of human peripheral blood-derived mast cells [19].

In summary, we have demonstrated that mast cells can be conveniently cultured from human hip bone marrow obtained at routine surgery. The cells demonstrate many features of tissue-derived mast cells and are therefore suitable for the study of human mast cell biology. In addition, we co-cultivated a second population of FcεRIα⁺ dendritic-like cells. The further characterization of these and their interaction with HBMMC is worthy of further study.

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