

Preliminary Report

Histamine release from human buffy coat-derived mast cells

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Abstract

Mast cells are unique immune cells that release a spectrum of chemical mediators contributing to the inflammatory symptoms of allergic disorders. Although mast cell biology has been extensively studied in the rodents, research on human mast cells is hampered by the lack of a convenient preparation source. This problem has now been addressed by culturing human mast cells from CD34⁺ progenitors. We have recently discovered that human buffy coat preparations from local blood banks are an abundant and convenient source of progenitors for culturing mature mast cells which express functional high affinity IgE receptors and contain histamine and tryptase in their granules. In the current study, we further characterize these buffy coat-derived mast cells by studying their responses to common mast cell secretagogues and stabilizers. Mature human mast cells were obtained by culturing isolated progenitors in methylcellulose containing stem cell factor (SCF), IL-3 and IL-6 for 6 weeks and subsequently in liquid medium containing SCF and IL-6 for another 6 to 8 weeks. Following sensitisation with human IgE, these cells released histamine dose-dependently upon activation by anti-IgE and calcium ionophores while compound 48/80 and substance P were relatively ineffective. When the effects of anti-asthmatic agents on anti-IgE-induced mediator release from these cells were compared, only the β_2 -adrenoceptor agonists and phosphodiesterase inhibitors produced dose-dependent inhibition but not cromolyn or nedocromil. In total, mast cells cultured from human buffy coat progenitors shared similar functional properties of MC_T subtype of mast cells found predominantly in human lung parenchyma and intestinal mucosa.

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Keywords: Buffy coat; Human mast cell culture; Histamine release**1. Introduction**

Mast cells are widely distributed throughout the body and are recognized as a major inducer of allergic and inflammatory reactions due to their high-affinity receptors for immunoglobulin E (IgE) and their contents of potent inflammatory mediators [1]. The immunolog-

ical activation of mast cells through cross-linking of the high affinity IgE receptors (Fc ϵ RI) initiates the release of preformed granular content, such as histamine, neutral proteases and proteoglycans within minutes of activation and is followed by the de novo synthesis of lipid mediators, such as cysteinyl-leukotrienes (cys-LTs) and prostaglandin (PG) D₂, and cytokines, such as TNF- α and IL-8 [2]. Studies using enzymatically dissociated tissue mast cells revealed at least two types of human mast cells based upon their granular content of proteases [3,4]. Mast cells in human lung parenchyma and intestinal mucosa predominantly contain tryptase only (MC_T) whereas mast cells in the skin and intestinal

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submucosa contain both tryptase and chymase (MC_{TC}) [5–7]. Furthermore, these two subtypes of human mast cells demonstrate varying responses to different activating stimuli and pharmacological modulators of mast cell activation [7,8]. However, more comprehensive research into the roles of mast cells in human physiology and diseases has been hampered by the lack of a convenient source providing sufficient number of purified mast cells. The recent development of mast cell culturing techniques attempted to overcome this problem by generating homogeneous population of mast cells from progenitor cells. The most common culturing methods for human mast cells involve initial promotion of colony growth of crude human hematopoietic cells by the fibroblast-derived mast cell growth factor, stem cell factor (SCF) or *c-kit* ligand and subsequently facilitation of mast cells maturation by additional cytokines such as IL-3, IL-6 and GM-CSF [9–11]. The current most frequent sources of mast cell progenitors for culturing human mast cells are fresh human peripheral blood and umbilical cord blood [10,12,13]. However, such sources are not always available and their uses always require special permissions. In contrast, human buffy coat preparations generated during the preparation of donated blood are readily available from blood banks and we have demonstrated that they can be a more convenient and abundant source of mast cell progenitors [14]. Since cord blood-derived mast cells are less responsive than adult peripheral blood-derived mast cells [15], we investigated the effects of common mast cell activators and inhibitors on histamine release from mast cells cultured from buffy coat progenitors to evaluate if they are as functionally comparable to human tissue mast cells as the peripheral blood-derived mast cells.

2. Materials and methods

2.1. Reagents

Microbead conjugated with monoclonal mouse anti-human antibodies against CD4, CD8, CD11b, CD14 or CD19 were purchased from Miltenyi Biotec (Bergisch Gladbach, Germany). Silica was from Immuno-Biological Labs., Gunma, Japan. Dulbecco's phosphate buffered saline, Iscove's modified Dulbecco's medium, fetal bovine serum, insulin–transferrin–selenium, 2-mercaptoethanol, penicillin–streptomycin, bovine serum albumin (BSA), human myeloma IgE were from Gibco-BRL, Gaithersburg, MD. Lymphocytes separation medium was from ICN Pharmaceutical, Costa Mesa, CA. Serum-free methylcellulose medium (METHOCULT™ SF^{BIT}) was from StemCell Technologies, Vancouver, BC, Canada. Human SCF, IL-6 and IL-3 were from PeproTech Asia, Rehovot, Israel. Human myeloma IgE for the passive sensitization of human culture mast cells was purchased from GIBCO BRL, Grand

Island, NY. 3-Isobutyl-1-methylxanthine (IBMX) and rolipram were purchased from Tocris, Avonmouth, UK. All other mast cell activating agents and pharmacological reagents were purchased from Sigma, St. Louis, MO.

2.2. Isolation of pluripotent stem cells and culture of mast cells from buffy coat

Fresh buffy coats of not more than 24 h old were obtained from the Hong Kong Red Cross and were cultured according to methods previously described [14]. Phagocytic cells were precipitated subsequent to incubation of the buffy coat with 1:10 volume of Silica (Immuno Biological Laboratories, Fujioka, Japan) for 30 min at 37 °C. Non-phagocytic mononuclear cells were separated by density-gradient centrifugation using Lymphocyte Separation Medium (Organon Teknika Corp. Durham, NC) according to the supplier's instruction. For concentrating CD34⁺ progenitor cells, CD4⁺ CD8⁺ CD11b⁺ CD14⁺ and CD19⁺ cells were removed from mononuclear cells using a magnetic separation column (Super Macs II, Miltenyi Biotec, Bergisch Gladbach, Germany) and a mixture of magnetic microbeads-conjugated with antibodies against CD4, CD8, CD11b, CD14 and CD19 (Miltenyi Biotec) according to the manufacturer's instructions.

Mononuclear cells eluted from the magnetic column were suspended in Iscove's modified Dulbecco's medium (GIBCO) supplemented with 1% insulin–transferrin–selenium (GIBCO), 5×10^{-5} M 2-mercaptoethanol (GIBCO), 1% penicillin+streptomycin (GIBCO) and 0.1% BSA (Sigma). The cell suspension was mixed well with serum-free Iscove's methylcellulose medium (Stem Cell Technologies Inc. Vancouver, BC, Canada) containing SCF (200 ng/ml), IL-6 (50 ng/ml) and IL-3 (5 ng/ml) and was then inoculated at 0.3 ml/well in the 24-well plates (Corning, NY, USA) at 37 °C in 5% CO₂. Every 10–14 days, 0.3 ml of fresh methylcellulose medium containing SCF (100 ng/ml)+IL-6 (50 ng/ml) without cells was layered over the methylcellulose cultures. After 6 weeks of culture, the methylcellulose was dissolved in PBS and the cells were resuspended and cultured in liquid medium containing SCF (100 ng/ml) and IL-6 (50 ng/ml) with 2% foetal calf serum (GIBCO) for another 6–8 weeks before harvesting for experiments.

2.3. Mast cell incubation and histamine assay

Prior to all experiments, buffy coat-derived mast cells were sensitized with human myeloma IgE (GIBCO BRL) by incubating with 0.1 µg/ml of human myeloma IgE for 1 h at 37 °C. After washing with full HEPES buffer (FHB: 137 mM NaCl, 5.56 mM glucose, 12 mM HEPES, 2.7 mM KCl, 0.4 mM NaH₂PO₄ and 1 mM CaCl₂ at pH 7.4) supplemented with 0.03% human albumin (HA), the sensitized mast cells were resuspended in prewarmed FHB/HA buffer and were further incubated for 10 min at 37 °C. In experiments studying the effects of mast cell stimuli, 200 µl of the prewarmed mast cell suspension ($0.5\text{--}3.0 \times 10^4$ cells) were added to tubes each containing 200 µl of secretagogue solution (anti-IgE, calcium

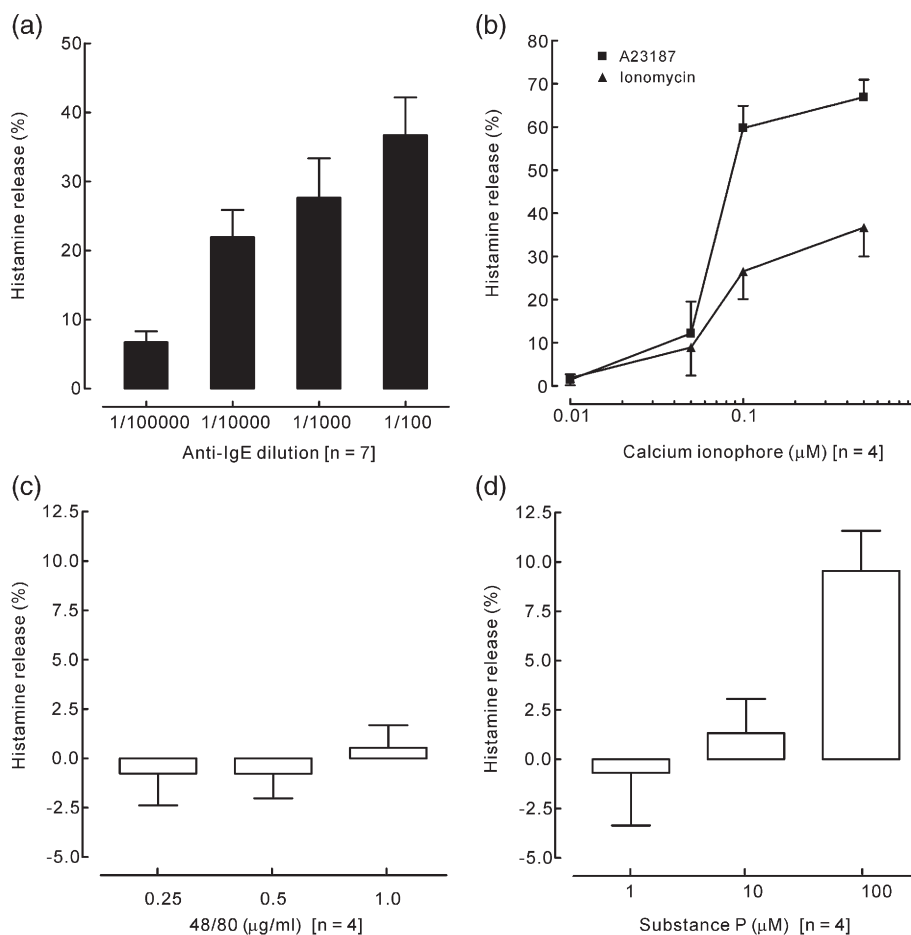


Fig. 1. Histamine release from mature mast cells cultured from buffy coat progenitors induced by (a) anti-IgE, (b) calcium ionophores, (c) compound 48/80 and (d) substance P. Mast cells were sensitized by incubation with myeloma IgE for 1 h at 37 °C prior to experiments. All results were corrected for spontaneous release ($7.4 \pm 4.0\%$ to $8.9 \pm 1.4\%$) and are mean $\pm$ S.E.M. for 4–7 experiments.

ionophore A23187, ionomycin, compound 48/80 and substance P) and were further incubated for 30 min at 37 °C. In experiments studying the effects of pharmacological agents on anti-IgE-induced mediator release, anti-IgE (1:1000 dilution) was added after preincubating the cells for 10 min with the agents. Cells incubated in buffer alone served as a measure of the spontaneous histamine release. Histamine release was stopped by addition of 600 μ l ice-cold buffer followed immediately by centrifugation ($430\times g$) for 5 min at 4 °C to separate the supernatants from cell pellets. Histamine contents in the supernatant and cell pellet fractions were assayed spectrofluorimetrically using a Bran+Luebbe AutoAnalyzer (Bran+Luebbe GmbH, Norderstedt, Germany) which chemically extracted histamine from the samples as described by Siraganian [16].

2.4. Expression of results and statistical analyses

The level of histamine release was expressed as a percentage of total cellular histamine whereas the activity of pharmacological agents was expressed as the percentage of the anti-IgE-

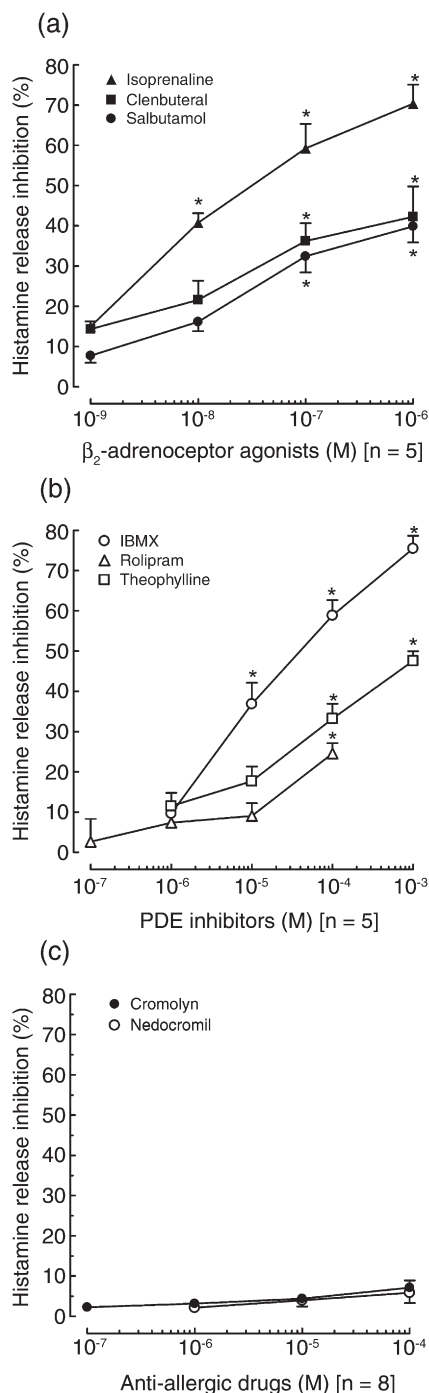
induced release which was inhibited in the presence of a pharmacological agent. The actual level of mediator release in the presence of a pharmacological agent were compared with the corresponding control value for statistical significance analysis using Student's *t*-test for paired data and values were considered significantly different at $p < 0.05$. All data are mean $\pm$ standard error of mean (S.E.M.) for *n* independent observations.

3. Results

3.1. Effects of mast cell activators on histamine release

The spontaneous histamine release of the buffy coat-derived mast cells in buffer alone was in general less than 10%. Subsequent to sensitization with human myeloma IgE, anti-human IgE antibody-induced dose-dependent release of histamine and $36.7 \pm 5.5\%$ of total cellular histamine was released at 1:100 dilution of anti-IgE (Fig. 1a). The calcium ionophores A23187 and ionomycin also dose-dependently induced histamine release from the buffy coat-derived mast cells (Fig. 1b).

66.9±4.0% and 36.7±6.7% of histamine release was induced by A23187 and ionomycin, respectively, at the highest concentration of 0.5 μ M tested. In contrast, no significant histamine release was induced by compound 48/80 and substance P, at concentrations of up to 1 μ g/ml and 10 μ M, respectively (Fig. 1c, d). Even at the very high concentration of 100 μ M, substance P could only induce 9.6±2.0% of histamine release.



3.2. Effects of pharmacological agents on anti-IgE-induced mediator release

For investigating the effects of pharmacological modulators, the level of anti-IgE-induced histamine release was controlled at around 35% by using 1:10,000 dilution of anti-IgE. The β -adrenoceptor agonists, clenbuterol, isoprenaline and salbutamol, suppressed anti-IgE-induced histamine release from anti-IgE-induced histamine release in a dose-dependent manner (10⁻⁹ M to 10⁻⁶ M) (Fig. 2a). At the maximum concentration of 10⁻⁶ M tested, isoprenaline, clenbuterol and salbutamol inhibited respectively 70.4±4.7%, 42.3±7.6% and 39.9±4.0% of histamine release. Similarly, the non-specific phosphodiesterase (PDE) inhibitors theophylline and IBMX as well as the specific type 4 PDE isoenzyme (PDE 4) inhibitor rolipram also dose-dependently inhibited anti-IgE-induced release of histamine and the levels of inhibition were 47.6±2.4%, 75.5±3.2% and 24.6±2.6%, respectively, at the highest concentrations tested (Fig. 2b). In contrast to β -adrenoceptor agonists and phosphodiesterase inhibitors, the prototype anti-allergic agent cromolyn and its congener nedocromil failed to significantly affect the anti-IgE-induced histamine release (Fig. 2c).

4. Discussion

Early studies on mast cells isolated from human tissues revealed that mast cells from different locations demonstrated different functional responses to various mast cell secretagogues and pharmacological agents [17]. Although all human tissue mast cells are responsive to activation via the Fc ϵ -RI receptors, only mast cells from human skin, intestinal submucosa and bladder are responsive to the polycationic stimuli compound 48/80 and substance P while mast cells from the lung and intestinal mucosa are unresponsive [18–21]. The discrepancy in responses is attributable to the heterogeneous populations of mast cells in these locations [22]. Mast cells containing both chymase and tryptase in their secretory granules (MC_{TC}) predominate in tissues which are activated by the polycationic compounds whereas mast cells containing tryptase only (MC_T) predominate in unresponsive tissues [5–7]. Over 90% of the buffy coat-derived mast cells contain only tryptase in their cytoplasmic granules as detected immunocytochemically

Fig. 2. Inhibitory effects of (a) β_2 -adrenoceptor agonists, (b) phosphodiesterase inhibitors (c) anti-allergic compounds on anti-IgE-induced histamine release from mature mast cells cultured from human buffy coat progenitors. Sensitized mast cells were incubated with one of the anti-asthmatic agents for 10 min prior to challenging with 1:1000 dilution of anti-IgE. Anti-IgE induced the release of 33.2±4.0% to 35.4±2.3% of histamine after correction for spontaneous release. Results are mean±S.E.M. for 5 experiments. **p*<0.05 when the levels of actual anti-IgE-induced histamine release in the presence of drugs were compared with the anti-IgE-induced release in buffer alone.

with anti-human tryptase and anti-human chymase antibodies as previously reported by us [14]. These buffy coat-derived mast cells are then expected to demonstrate similar functional characteristics of MC_T mast cells.

Interaction of anti-IgE antibody with IgE bound to Fc_εRI on the cell surface initiates a complex series of phosphate transfer events via the activation of various tyrosine kinases and consequently the activation of phospholipase C and intracellular calcium increase which is essential for the exocytosis of histamine from cytoplasmic granules [2]. Calcium ionophores increase intracellular calcium concentration by transporting calcium from the outer environment into the cells and hence induce mediator release in mast cells from all human tissues studied [23]. As demonstrated by their dose-dependent response to anti-IgE and calcium ionophores, the buffy coat-derived mast cells possess functional Fc_εR-I receptors and complete biochemical machinery for exocytosis which are shared by all tissue mast cells. Furthermore, the maximum level of anti-IgE-induced histamine release of $36.7 \pm 5.5\%$ is comparable to those reported for human lung mast cells and peripheral blood-derived mast cells [14,17]. The failure of the buffy coat-derived mast cells to respond to compound 48/80 and physiological concentrations of substance P suggested that they resemble the MC_T cells isolated from human lung and intestinal mucosa.

Histamine release from human tissue mast cells in general are suppressed by agents which elevate intracellular level of cyclic AMP and the most studied compounds include β -adrenoceptor agonists and phosphodiesterase inhibitors [24]. In the current study, the β -adrenoceptor agonists, isoprenaline, salbutamol and clenbuterol are potent inhibitors of immunologically stimulated histamine release from the buffy coat-derived mast cells. The inhibition of mediator release by the selective β_2 -adrenoceptor agonists salbutamol and clenbuterol suggests that, similar to human tissue mast cells and peripheral blood-derived mast cells, the β_2 -adrenoceptor is the primary β -adrenoceptor expressed in our cultured mast cells [25,26]. While both non-specific phosphodiesterase (PDE) inhibitors inhibited anti-IgE-induced histamine release from buffy coat-derived mast cells, IBMX was more potent than theophylline. The concentrations that caused 50% inhibition were approximately around 0.04 and 1 mM for IBMX and theophylline respectively in the current study as estimated from Fig. 2b and were comparable with the respective values of 0.25 and 1.25 mM reported for human lung mast cells [27,28]. Although the maximum concentration of rolipram tested was only 10^{-5} M due to its low solubility, the concentration–response curve of rolipram resembles that of theophylline within the same concentration range, suggesting the

existence of PDE 4 isoforms in our buffy coat-derived mast cells.

Although cromolyn and nedocromil possess mast cell-stabilizing properties which are highly tissue and species specific in the rat, they generally demonstrate weak and variable degree of inhibition on mediator release from human tissue mast cells [19,29]. Similarly, both cromolyn and nedocromil failed to significantly reduce the level of anti-IgE-induced histamine release in our cultured human mast cells. Although the inhibitory effects of cromolyn and nedocromil are known to undergo rapid tachyphylaxis, their lack of significant inhibitory action in the current study is not due to the 10 min preincubation treatment employed since no significant effect was observed even when these anti-allergic compounds were added to the buffy coat-derived mast cells at the time of anti-IgE challenge in our preliminary studies (results not shown).

The current investigation has clearly demonstrated that functionally viable human mast cells can be cultured from progenitors isolated from buffy coat preparations which are indeed a more abundant and less restrictive source of human mast cell progenitors than the earlier sources such as fetal liver cells, umbilical cord blood and fresh adult peripheral blood. Apart from resembling the MC_T mast cells in human lungs and intestinal mucosa with reference to their expression of human tryptase as the only granule-associated neutral protease, these buffy coat-derived mast cells are further demonstrated to be similarly modulated by various mast cell secretagogues and pharmacological agents as mast cells isolated from human lung parenchyma and intestinal mucosa as well as those cultured from fresh human peripheral blood [13]. Buffy coat-derived mast cells can hence be a more convenient alternative to mast cells derived from the umbilical cord blood and adult peripheral blood as a useful model for future studies on human mast cell biology and the screening of potential anti-allergic compounds.

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