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Mast cell subtypes from human lung tissue: their identification, separation, and functional characteristics

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Abstract

The contribution of mast cell subtypes and their different mediators to the pathogenesis of chronic obstructive lung diseases (COLD) has not yet been established. In the present study, enzymatic digestion, centrifugal elutriation and Percoll gradient centrifugation were used to obtain two populations of mast cell subtypes from human lung tissue. Mast cell subtypes were challenged with anti-human IgE, propranolol, compound 48/80, or opsonized zymosan. Both subtypes were able to release histamine, but differed in the amount of the amine released. Only the formalin-sensitive and alcian blue-positive type (FS-AB) released histamine on challenge with opsonized zymosan. The same subtype was able to release leukotriene C_4 (LTC_4) after challenge with anti-human IgE. The other subtype, the formalin-insensitive and alcian blue-positive type (FI-AB), did not respond to opsonized zymosan and did not release LTC_4 after challenge with anti-human IgE. Stimulation with propranolol or compound 48/80 did not release histamine from the FS-AB mast cells while the FI-AB mast cells released only about 10% of their histamine content upon challenge with these secretagogues.

Introduction

Mast cells may play an important role in the pathogenesis of chronic obstructive lung diseases (COLD) such as asthma [1, 2]. Recent investigations have revealed the existence of mast cell heterogeneity [3, 4] and subpopulations of mast cells have been differentiated on the basis of histochemical characteristics [3, 5, 6]. In the present study, a method is provided for the separation from human lung tissue of mucosal-like mast cells (MMC), which are formalin-sensitive and alcian blue-positive (FS-AB), from connective tissue-like mast cells (CTMC), which are formalin-insensitive and alcian blue-positive (FI-AB). The contribution of these mast cell subtypes and their mediators to the pathogenesis of COLD remains to be established.

Materials and methods

Human lung tissue was obtained at thoracotomy, mainly performed for the resection of bronchial carcinomas. Mast cells were isolated from the tissue as described previously [7]. In short, the isolation procedure consisted of the following steps. Macroscopically normal lung tissue was dissected free of major airways and blood vessels. The tissue was fragmented with scissors and suspended in RPMI-1640 containing 10% heat-inactivated fetal calf serum. Subsequently, the lung fragments were washed intensively to remove blood cells. The lung fragments were digested four times by collagenase and thermolysin at 37 °C for 30 min per incubation step. The dispersed cells were separated into different fractions by centrifugal elutriation. Using a constant buffer flow of 20 ml

per minute and decreasing rotation-speeds from 3000 rpm to 1200 rpm, fractions of 150 ml each were collected (4 °C). The cells in the elutriation fractions obtained at 2100 rpm and 1800 rpm were layered on a discontinuous Percoll gradient with densities of 1.050, 1.060, 1.072, 1.082 and 1.100 g/ml and centrifuged at room temperature (400 × *g*, 20 min). Cells were collected and washed twice with Tyrode's buffer to remove the Percoll.

Histochemical characterization

Air-dried cytocentrifuge smears were stained with May Grunwald-Giemsa stain. Histochemical characterization of mast cell subtypes was performed by staining smears with alcian blue-safranin O after fixation in Carnoys fluid (60% ethanol, 30% chloroform, 10% acetic acid) or 10% formol-saline.

Functional characterization

Functional characterization was performed by *in vitro* stimulation. Aliquots of isolated mast cell subtypes (1.5×10^4 mast cells/ml) were challenged by incubation with anti-IgE (diluted up to 1:100), propranolol (3.4×10^{-4} *M*) or compound 48/80 (1 μg/ml) for 0.5, 1 or 5 minutes at 37 °C. Alternatively they were challenged with opsonized zymosan (5 mg/ml) [8] for 1, 5, 10 and 20 min at 37 °C. Mediator release was stopped by centrifugation at 250 × *g* for 3 min at 4 °C. The supernatant was removed and assayed for histamine and leukotriene C_4 (LTC_4) content. Histamine content was assayed by a double isotope assay (^3H, ^{14}C) [9]; LTC_4 was detected by a ^3H-radioimmunoassay (New England Nuclear, Boston, USA) [10].

Results

Enzymatic digestion of lung tissue resulted in a yield of $15.0 \pm 1.5 \times 10^6$ cells per gram wet tissue (mean ± SEM, $n = 10$). This cell population consisted of 2.1 ± 0.4% mast cells, of which 66 ± 8% were FS-AB cells and 34 ± 8% FI-AB cells. After centrifugal elutriation, mast cells were found only in the fractions obtained at 2700, 2100 and 1800 rpm. The fractions 2700, 2100 and 1800 contained 2.0 ± 0.4, 4.3 ± 1.6, and 4.5 ± 2.1% mast cells, respectively. FS-AB mast cells constituted respectively 100 ± 0, 74 ± 11, and 40 ± 11% of the total mast cell population. After gradient centrifugation, mast cells were obtained in all bands. The mast cell content of these bands was respectively. 0.8 ± 0.2, 4.0 ± 1.2, 9.0 ± 2.5, 15.3 ± 3.7, and 8.4 ± 3.0% for increasing density. FS-AB mast cells constituted 11 ± 8, 59 ± 18, 82 ± 6, 93 ± 2, and 94 ± 1% of these mast cell populations. So, FI-AB mast cells were predominantly found at the lower densities. The histamine contents of the mast cells were 1.34 ± 0.15 pg/FS-AB cell and 4.17 ± 0.38 pg/FI-AB cell ($p < 0.001$, $n = 10$).

After challenge with anti-human IgE, a time and concentration dependent release of histamine and LTC_4 was observed. On anti-IgE (1:25 dilution) stimulation there was a significant difference ($p < 0.05$, $n = 10$) in histamine release between the mast cell subtypes (Table 1). FI-AB cells showed a higher histamine release than FS-AB cells (59 ± 3% vs 33 ± 5%). Only FS-AB mast cells showed histamine release on challenge with opsonized zymosan (72 ± 5%), and LTC_4 release on challenge with anti-IgE. After 1 min, 169 ± 35 pg LTC_4 per 1.5×10^4 mast cells was released. The amount of LTC_4 after 5 min was 69 ± 17 pg per 1.5×10^4 cells. Only FI-AB mast cells released histamine, albeit in small amount, on stimulation with propranolol or compound 48/80 (Table 1).

Table 1
Release of histamine and leukotriene C_4 (LTC_4) after challenge with different secretagogues of two subtypes of mast cells isolated from human lung tissue. The release is expressed as a percentage of the total histamine content and as pg LTC_4 per 1.5×10^4 mast cells. For concentrations of secretagogues, see text.

Stimulant	Inc. time (min)	Mast cell subtype	
		FI-AB	FS-AB
Histamine release (%)			
Anti-IgE	5	59 ± 3	33 ± 5
Propranolol	5	13 ± 8	7 ± 3
Compound 48/80	5	11 ± 8	5 ± 5
Opsonized zymosan	20	3 ± 1	72 ± 5
LTC_4 (pg/1.5×10^4 mast cells)			
Anti-IgE	1	–	169 ± 35
	5	–	69 ± 17

Discussion

We have demonstrated that in human lung tissue at least two types of mast cells can be identified on the basis of histochemical properties, histamine contents, and functional characteristics. Furthermore, we were able to separate these mast cell subpopulations based on their difference in density. The difference in behaviour between both subtypes can be compared with some characteristics of rodent CTMC and MMC, such as the lack of response to challenge with compound 48/80 and LTC_4 release on stimulation with anti-IgE of FS-AB cells compared with MMC [2, 11]. The existence of different mast cell subtypes with their specific properties means that they may contribute to the pathogenesis of COLD in a different manner. Further studies are now in progress to investigate these contributions.

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