

Cultured Human Mast Cells Are Heterogeneous for Expression of the High-Affinity IgE Receptor FcεRI

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Key Words

Anti-IgE · CD63 · CD203c · Cultured human mast cells · FcεRI · IL-4 · Histamine · Mast cell phenotype

Abstract

Objective: We determined the density of FcεRI on mast cells cultured from cord (CBMC) and peripheral blood (PBMC) and studied the kinetics of the response through FcεRI. **Methods:** Mast cells were cultured from CD133+ progenitors from peripheral or cord blood. FcεRI was stabilized by culture with 2 μg/ml IgE. Cells were activated by addition of anti-FcεRI antibody (1 ng/ml–10 μg/ml). Maximal activation, sensitivity, and cooperativity were determined. **Results:** All cultures were homogeneous for tryptase and metachromasy. All cells expressing FcεRI could be activated by cross-linking FcεRI to upregulate CD63. PBMC bind 203,000 molecules of IgE/cell. Stabilization of FcεRI with IgE doubled the number of CD63+ cells ($p = 0.0001$) and increased the sensitivity (from 0.083 to 0.013 μg/ml anti-FcεRI) and the slope factor (from 10.8 to 68) of PBMC but not of CBMC. Anti-IgE reversed these effects ($p = 0.0002$) but did not reduce activation levels below that of cell lines not stabilized with IgE. **Conclusion:** Baseline expression of FcεRI is independent of anti-IgE. The fraction of PBMC that binds high levels of IgE can be activated through FcεRI.

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Introduction

Classification of mast cells depending on localization as mucosal or connective tissue mast cells [1, 2] has been questioned by early experimental evidence of the plasticity of mast cells depending on the local environment [3]. The present classification depends on expression of chymase [4]. Recent work in the mouse [5] and man [6] highlights that the classification should include association of function and phenotype.

Expression of the high-affinity IgE receptor (FcεRI) was shown to vary considerably by anatomical microlocation [6]. Transcription of FcεRI is directly regulated by IL-4 [7]. That leads to a higher density of FcεRI on both basophil granulocytes [8] and mast cells [9].

CD63 [10] and CD203c [11] have been used as markers for basophil activation. They have different kinetics of translocation to the cell surface [11, 12] but are similar in clinical predictive value in basophil activation tests [13]. Both markers are upregulated on activated mast cells [14].

Omalizumab (Xolair®; Novartis, Basel, Switzerland), an anti-IgE (aIgE) antibody, reduces the circulating free IgE and FcεRI density on basophil granulocytes and mast cells [9, 15].

We recently compared mast cells cultured from cord blood (CBMC) and peripheral blood (PBMC) [16, 17]. Here we investigated the regulation of expression of FcεRI on PBMC and CBMC.

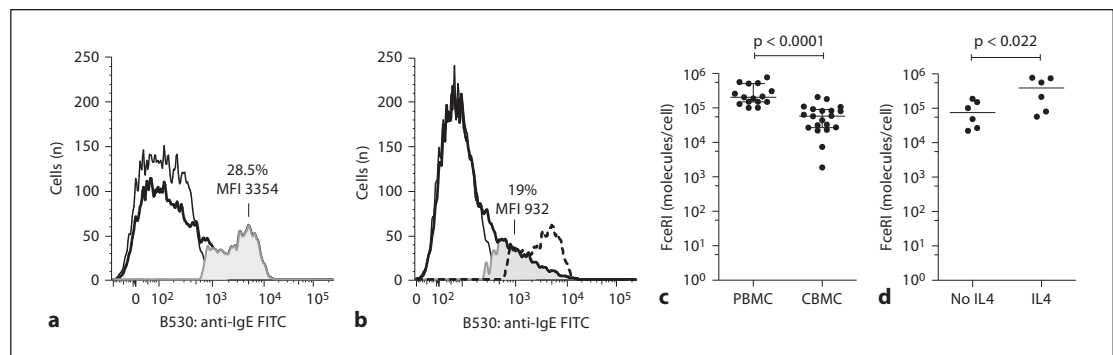


Fig. 1. IgE binding to mast cells. Representative data from PBMC (28.5% positive) (**a**) and CBMC (19% positive) (**b**). Cells cultured without IgE are a thin black line. Cells cultured with IgE are a thick black line. FcεRI-expressing, IgE-binding cells identified by FDG are indicated by a gray line and area. The black stippled line

in the CBMC pane is a trace of PBMC FcεRI. **c** Expression of FcεRI on human PBMC or CBMC. **d** Culture with IL-4 (10 ng/ml) for 2 weeks enhances FcεRI expression (IgE binding) on mast cells. Dots represent individual mast cell lines tested.

Methods

Mast Cell Culture

CD133+ progenitors purified from cord blood or buffy coats were cultured as described [16, 17]. Metachromasy, chymase, and tryptase expression and histamine release were evaluated [17]. Recombinant human IL-4 (PeproTech, London, UK) (10 ng/ml) was added to selected lines in complete medium (CM; Stem Span; Stem Cell Technologies, Vancouver, B.C., Canada) with 5% fetal bovine serum (GIBCO, Grand Island, N.Y., USA) and SCF and IL-6 (both from R&D Systems, Abingdon, UK) for a further 2 weeks to mature them [14]. At 7 or 9 weeks, mast cell lines were split. One well served as control. Monoclonal human/murine chimeric recombinant IgE (2 µg/ml) was added to 3 wells. aIgE (4 µg/ml) was added to 1 well with IgE and an antibody specific for CD3; OKT-3 (Janssen-Cilag, Birkerød, Denmark) (4 µg/ml) was added to the last well as an irrelevant control. Lines were cultured for 2 days.

Density of FcεRI

The density of FcεRI was determined using a QIFIKIT (catalog No. K0078; Dako, Glostrup, Denmark). Experimental details in MIFlowCyt format [18] for all experiments can be obtained from the author. Wells cultured for 2 days with 2 µg/ml IgE and a well without IgE were washed twice in PBS at 4°C. Both wells were incubated with aIgE (catalog No. 555894; Pharmingen, San Jose, Calif., USA). The FcεRI+ population was determined by frequency difference gating (FDG) [19] (fig. 1). The median expression and the interquartile range (IQR) measured on cultures without IgE was 10,318 (IQR 4,798–20,297) MESF.

Mast Cell Activity

Signaling through FcεRI was evaluated by culturing 100 µl of a mast cell line (approximately 50,000 cells) in CM with anti-FcεRI antibody CRA 1 (catalog No. 334602; Biolegend, San Diego, Calif., USA) in 5 log dilutions from 10 µg–1 ng/ml for 30 min at 37°C. The preparation was then incubated with antibody to CD63 (catalog No. 312004; Biolegend) and initially also with an antibody to CD203c (IM3575; Beckman Coulter). Cells were analyzed

on a FACS Canto II. Most lines contained a population of cells that expressed high levels of CD63 before stimulation (fig. 2i). The net fraction of activated mast cells was plotted against the concentration of anti-FcεRI added.

Statistics

Normally distributed data was compared with a t test. Data not normally distributed was compared using a Wilcoxon signed-rank test or a Mann-Whitney test. Correlation of normally and nonnormally distributed data was established by Pearson's and Spearman's methods, respectively. $p < 0.05$ was considered statistically significant.

Results

Among 11 lines, 3 expressed significant levels of FcεRI after 7 weeks. In the second series ($n = 6$), cultured $\pm$ IL-4, lines cultured with IL-4 expressed FcεRI. Lines not expressing FcεRI expressed high levels of CD63 and CD203c without stimulation. In the last series all lines ($n = 15$) were cultured with IL-4.

PBMC Bind More IgE than CBMC

Despite the apparent homogeneity for metachromasy and tryptase expression (both 100%), mast cell lines derived from peripheral blood and cord blood were not homogeneous for binding of IgE. Chymase expression ($<2\%$) was considered negative. The discrete population of cells with a common FcεRI/IgE density developed only in PBMC, suggesting that PBMC appear to be more differentiated than CBMC (fig. 1a, b). The density of IgE binding through FcεRI was evaluated on the positive fraction of each mast cell line.

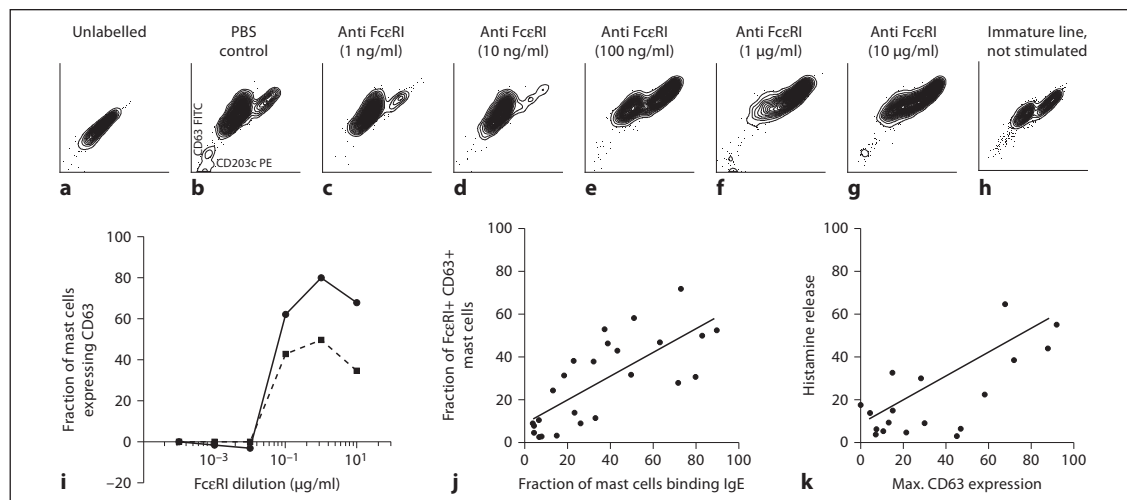


Fig. 2. Mast cell activation test. **a** Unlabelled mast cells. **b–g** Mast cell activation test. **h** An immature cell line that is unresponsive to FcεRI cross-linking contains a population of cells with a high surface expression of CD63 and CD203c at rest. **i** Percent of mast cells positive for CD63 (solid line) and CD203c (stippled line) against the anti-FcεRI concentration; from this plot, the maximal

activation, LC50, and slope coefficient are determined. **j** Correlation of fraction of cells binding anti-IgE with fraction of cells activated to express CD63 through FcεRI cross-linking ($r = 0.7800$, $n = 25$). **k** Correlation of fraction of histamine released with cells expressing CD63 through FcεRI cross-linking ($r = 0.5338$, $n = 18$).

PBMC bound a median of 203,000 IgE molecules/cell (IQR 140,000–517,000; $n = 17$) whereas CBMC bound 57,800 IgE molecules/cell (IQR 26,850–89,600; $n = 20$) ($p < 0.0001$) (fig. 1c). In paired experiments, mast cell lines cultured with IL-4 for 2 weeks doubled the number of IgE molecules bound per cell (fig. 1d) ($n = 6$, $p < 0.022$). As only a fraction of mast cell lines bound IgE, we investigated expression of FcεRI through a functional test.

Cross-Linking of FcεRI on Mast Cells Upregulates CD63 and CD203c

Mast cell lines incubated with anti-FcεRI antibody from 1 ng/ml to 10 μg/ml expressed CD63 and CD203c in a dose-dependent fashion (fig. 2a–h) with a characteristic bell-shaped curve (fig. 2i).

The fraction of mast cells expressing FcεRI at 7–10 weeks correlated with the fraction of mast cells expressing CD63 ($n = 29$, $p < 0.0001$) (fig. 2j) and with mast cell histamine release after stimulation with IgE/aIgE at 7 weeks ($n = 20$, $p = 0.0014$) (fig. 2k). The fraction of mast cells expressing CD63 correlated with the density of FcεRI on mast cells ($n = 29$, $p < 0.0001$).

Lines that could not be activated by cross-linking of FcεRI after 7 weeks of culture contained a significant population of cells that expressed CD63 and CD203c in the absence of all stimulation (fig. 2h), similar to the small population seen before stimulation in responsive mast

cell lines (e.g. fig. 2b). Culture for 2 weeks with IL-4 reduced the size of this population. In paired experiments, culture with IL-4 quadrupled the fraction of cells that could be activated ($n = 6$, $p = 0.017$, not shown).

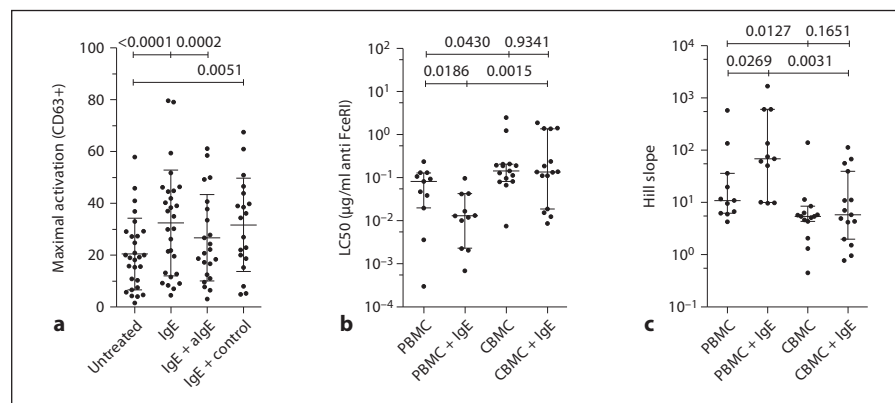
Anti-IgE Limits IgE Stabilization of FcεRI but Not Intrinsic Expression of FcεRI

The number of cells activated by cross-linking FcεRI was doubled by stabilization of FcεRI with IgE for 2 days ($n = 28$, $p = 0.0001$) (fig. 3a). Addition of aIgE neutralized this increase ($p = 0.0002$) but did not reduce the fraction of activated cells to below that of the untreated mast cell line.

Kinetic Analysis of Mast Cell Line Responses

As a measure of sensitivity the point of inflection (LC50) of the sigmoid curve fitted to the data correlated significantly with the density of FcεRI/IgE ($n = 20$, $p = 0.003$). As expected from the higher density of FcεRI/IgE, PBMC were nearly twice as sensitive as CBMC ($p = 0.043$) and were 10-fold more sensitive when FcεRI was stabilized with IgE ($p < 0.002$) (fig. 3b). Stabilization of FcεRI with 2 μg/ml IgE increased the sensitivity of PBMC from a median of 0.083 μg/ml anti-FcεRI (IQR 0.02–0.23) to 0.013 μg/ml anti-FcεRI (IQR 0.0023–0.042, $n = 11$, $p = 0.019$). This was not seen with CBMC.

Fig. 3. Effect of anti-IgE on mast cell FcεRI expression. **a** The effect on activation through FcεRI was measured as the maximal upregulation of CD63. **b** Data from **a** normalised to untreated lines. LC50, a measure of basophil sensitivity, decreased significantly as PBMC were stabilized by IgE, but not when IgE was added to CBMC. **c** The slope factor could be increased significantly in PBMC but not in CBMC.



The slope factor of the sigmoid dose-response curve of CD63 to anti-FcεRI cross-linking assessed cooperativity. While the slope of CBMC remained between 5 and 6 upon addition of IL-4 or IgE, the slope factor of the response of PBMC increased from 10.79 (IQR 6.19–35.88) by stabilization with IgE to 68.14 (IQR 9.98–597.5, $n = 11$, $p = 0.027$) (fig. 3c).

Discussion

Mast cells cultured from peripheral blood progenitors differentiate to a homogeneous population that binds 203,000 IgE molecules/cell, whereas CBMC by the same protocol do not differentiate into a population homogeneous for FcεRI expression and only bind 57,800 molecules IgE/cell. PBMC appear to be more terminally differentiated than CBMC as they expressed more molecules of FcεRI and were more responsive to modulation of sensitivity and the slope factor by IL-4, IgE, and aIgE. The effect of aIgE does not extend to IgE-independent upregulation of FcεRI. CD63 upregulation on activated mast cells correlates with histamine release. Assuming that the QIFIKIT measurement is the result of 1:1 interactions between IgE, aIgE, and FITC-labeled anti-Ig, and as the interaction of FcεRI and IgE is monomeric, this may translate directly to molecules of FcεRI expressed on the cell surface. The density of FcεRI on mast cells is likely to be heterogeneous as the phenotype of mast cells is heterogeneous and highly dependent on the local environment [1, 2]. The density of FcεRI on basophils [8, 15, 20] and mast cells [9] is regulated by IgE. The density of FcεRI on mast cells and basophil granulocytes is primarily determined by the stabilizing effect of IgE on the surface-bound receptor [21]. On human basophils, removal of stabilizing IgE reduces both surface and total cellular FcεRI [22] by

endocytosis for degradation or reexposure on the cell surface [23]. IL-4 induces an allergic phenotype in mast cells [14, 17] and directly induces FcεRI gene expression [7].

CD63 and CD203c are expressed on resting, unresponsive cells that do not yet express FcεRI but express tryptase, are metachromatic, and contain high levels of histamine (fig. 2h). CD63 is a tumor marker associated with early-stage, noninvasive cancer [24]. Expression of CD203c has been shown to be elevated on neoplastic mast cells and to be induced by FcεRI-mediated activation of mast cells [25].

CD63 and CD203c have previously been used as markers for allergic type I activation through FcεRI on basophils [10, 13, 26] and mast cells [14]. We noted that the fraction of mast cells binding IgE (fig. 1c) and the fraction maximally activated (fig. 2i) correlated (fig. 2j). On this basis we propose that all mast cells responding to FcεRI cross-linking do express FcεRI and, conversely, that there is a population of mature mast cells in the culture that does not express FcεRI. As responsive cultures are homogeneous for CD63 and CD203c expression in the absence of stimuli, tryptase staining, and metachromasy and have a high histamine content, these cells may be a distinct population of mature FcεRI⁺ mast cells.

The heterogeneity of mast cells has until now been described in terms of protease content rather than in terms of function [2].

Mast cells in the human lung are very heterogeneous, and this heterogeneity extends to expression of FcεRI [6]. Mast cell density in the human lung increases from the central airway to the peripheral airway, but expression of FcεRI on mast cells decreases from the central airway to the peripheral airway, suggesting that there is a large fraction of mast cells that may not express FcεRI. FcεRI expression would be a good candidate marker for an allergic mast cell in an alternative nomenclature for mast cell heterogeneity.

aIgE inhibits stabilization of FcεRI by IgE but does not affect intrinsic, basal expression of FcεRI that is regulated through IL-4 or other factors independently of stabilization by IgE. In addition to modulating FcεRI stability through aIgE, modulation of the amount of accessible FcεRI may be a useful target for long-term allergy control.

Both sensitivity and cooperativity increased as FcεRI was stabilized on PBMC but not on CBMC. CBMC have a lesser allergic phenotype than PBMC cultured with the same protocol in the same laboratory [16, 17] as they express less FcεRI and appear to be less responsive to the stabilizing effects of IgE.

Expression of FcεRI on cultured human mast cells characterizes a functionally defined subset of mast cells cultured from peripheral blood that is enhanced by IL-4 and stabilized by IgE and manifests itself through up-regulation of CD63 and CD203c.

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