Immunophenotyping and Functional Analysis of Purified Human Uterine Mast Cells

By Chao-Bo Guo, Anne Kagey-Sobotka, Lawrence M. Lichtenstein, and Bruce S. Bochner

Human mast cells have been purified from uterine tissues, and their surface marker profile and function have been evaluated as part of ongoing studies of mast cell heterogeneity. Using a panel of antibodies, purified uterine mast cells (UMC; 81% ± 7% purity, n = 10) were analyzed by immunofluorescence and flow cytometry for surface expression of various antigens. Consistent with previous analyses of mast cells from other tissues, UMC expressed HLA class I, IgE, c-kit receptor, CD9, CD33, CD43, CD45, and CD54, while CD11a, CD11b, CD14, CD16, CD23, and CD64 were not detected. Unlike other mast cells, UMC expressed CD11c/CD18 (p150,95) and CD32 (FcγRII). Additional antigens not previously studied on mast cells included the selectin LECAM-1 (Leu-8) and several β1 and β3 integrins; expression of very late activation antigen-4 (VLA-4) (CD49d/CD29), VLA-5 (CD49e/CD29), and the vitronectin receptor (CD51/CD61) was seen. Functional studies showed that treatment of human umbilical vein endothelial cells with interleukin-1 (5 ng/mL for 4 hours) resulted in a twofold to threefold increase in adhesiveness for UMC. Purification procedures did not alter histamine release responses to anti-IgE or the calcium ionophore A23187, and treatment of UMC with an anti-CD32 monoclonal antibody (IV.3) did not induce histamine release or alter anti-IgE-induced release. These data suggest that UMC may possess unique phenotypic characteristics, and support the concept of mast cell heterogeneity.

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necessary, cells were subsequently incubated with PE-conjugated polyclonal goat antimouse IgG (Tago, Burlingame, CA). Using an EPICS Profile Flow Cytometer (Coulter), initial gating was performed using forward and side scatter to eliminate debris and identify a uniform population of mast cells. A total of 1,000 to 5,000 mast cell events were collected, and histograms and the percentage of cells with a fluorescence intensity ≥ 95% of all control events (percent positive) were determined.

### Table 1. Phenotyping of Human UMC

<table>
<thead>
<tr>
<th>Surface Antigen</th>
<th>MoAb (isotype)</th>
<th>% Positive ± SEM</th>
<th>Expressed on Other Human Mast Cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA class I</td>
<td>W6/32 (IgG2a)</td>
<td>3 100 ± 0</td>
<td>Yes</td>
</tr>
<tr>
<td>c-kit receptor</td>
<td>YB5.88 (IgG1)</td>
<td>6 78 ± 9</td>
<td>Yes</td>
</tr>
<tr>
<td>IgE</td>
<td>Anti-IgE (polyconal)</td>
<td>6 72 ± 8</td>
<td>Yes</td>
</tr>
<tr>
<td>LE CAM-1</td>
<td>Leu-9 (IgG2a)</td>
<td>2 2 ± 1</td>
<td>?</td>
</tr>
<tr>
<td>CD9 (p24)</td>
<td>ALB-6 (IgG1)</td>
<td>3 97 ± 1</td>
<td>Yes</td>
</tr>
<tr>
<td>CD11a (LFA-1α)</td>
<td>MMH24 (IgG1)</td>
<td>4 18 ± 11</td>
<td>No</td>
</tr>
<tr>
<td>CD11b (Mac-1α)</td>
<td>HS44 (IgG1)</td>
<td>4 14 ± 5</td>
<td>No</td>
</tr>
<tr>
<td>CD11c (p150,95a)</td>
<td>SHCL-3 (IgG2b)</td>
<td>4 75 ± 12</td>
<td>No</td>
</tr>
<tr>
<td>CD14 (p55)</td>
<td>Leu-M3 (IgG2b)</td>
<td>3 7 ± 1</td>
<td>No</td>
</tr>
<tr>
<td>CD16 (FcγRIII)</td>
<td>3G8 (IgG1)</td>
<td>4 9 ± 3</td>
<td>No</td>
</tr>
<tr>
<td>CD18 (β2 integrin)</td>
<td>HS2 (IgG1)</td>
<td>4 72 ± 7</td>
<td>No</td>
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<td>CD23 (FcεRI)</td>
<td>MMH6 (IgG1)</td>
<td>4 6 ± 3</td>
<td>No</td>
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<td>CD29 (β1 integrin)</td>
<td>K20 (IgG2a)</td>
<td>2 99 ± 2</td>
<td>?</td>
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<tr>
<td>CD32 (FcγRII)</td>
<td>IV.3 (IgG2b)</td>
<td>5 89 ± 5</td>
<td>No</td>
</tr>
<tr>
<td>CD33 (gp67)</td>
<td>My9 (IgG2b)</td>
<td>1 97</td>
<td>No</td>
</tr>
<tr>
<td>CD43 (leukosiaiin)</td>
<td>Leu-22 (IgG1)</td>
<td>2 98 ± 2</td>
<td>Yes</td>
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<tr>
<td>CD45 (LCA)</td>
<td>KC66 (IgG1)</td>
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<td>Yes</td>
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<tr>
<td>CD49b (VLA-2α)</td>
<td>G19 (IgG1)</td>
<td>3 7 ± 3</td>
<td>?</td>
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<tr>
<td>CD49d (VLA-4α)</td>
<td>HP2/1 (IgG1)</td>
<td>4 97 ± 2</td>
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<td>CD49e (VLA-5α)</td>
<td>SAM-1 (IgG1)</td>
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<td>?</td>
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<tr>
<td>CD49f (VLA-6α)</td>
<td>GoH-3 (IgG2a)</td>
<td>1 5</td>
<td>?</td>
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<tr>
<td>CD51 (vitronectin α)</td>
<td>AMF-7 (IgG1)</td>
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<tr>
<td>CD54 (ICAM-1)</td>
<td>84H10 (IgG1)</td>
<td>4 87 ± 10</td>
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<tr>
<td>CD61 (β3 integrin)</td>
<td>S2.21 (IgG1)</td>
<td>3 75 ± 15</td>
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<tr>
<td>CD64 (FcγRII)</td>
<td>32.2 (IgG1)</td>
<td>3 20 ± 9</td>
<td>No</td>
</tr>
</tbody>
</table>

Abbreviations: N, number of experiments; ?, not previously reported; LCA, leukocyte common antigen.  
*Based on previously published data. 

**RESULTS**

**Phenotypic analysis of purified human UMC.** Using immunofluorescence and flow cytometry, we determined the surface expression of a variety of antigens (Table 1). Consistent with previous reports examining surface markers on human lung mast cells (summarized in Table 1), UMC expressed HLA class I, IgE, the c-kit receptor (recognized by YB5.88), CD9, CD33, CD43, CD45, and CD54; surface expression of CD11a, CD11b, CD14, CD16, CD23, and CD64 was not detected. In contrast to previous reports with other mast cells, UMC expressed both the α and β subunits of the integrin p150,95 (CD11c, CD18), as well as FcγRII (CD32). Both the CD11c and CD32 antigens were detected using two different MoAbs. Although not previously examined on mast cells, additional surface
molecules present on UMC included the β1 integrins very late activation antigen-4 (VLA-4) (CD49d/CD29, a counter-receptor for vascular cell adhesion molecule-1 [VCAM-1]) and VLA-5 (CD49e/CD29, a receptor for fibronectin), as well as one of the β3 integrins referred to as the vitronectin receptor (CD51/CD61). Expression of the selectin LECAM-1 (recognized by the Leu-8 antibody) and the α subunits of the collagen receptor (CD49b) and the laminin receptor (CD49f) was not detected. Representative flow cytometric histograms for surface markers present on human UMC are shown in Fig 2.

Adherence of human UMC to cytokine-activated endothelial cells. Treatment of human umbilical vein endothelial cells with IL-1 is known to induce the expression of a number of adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1), endothelial cell adhesion molecule-1 (ELAM-1), and VCAM-1.23-26 Experiments were performed to determine whether such treatment resulted in enhanced adhesiveness for UMC. In two separate experiments, treatment of endothelial cells for 4 hours with 5 ng/mL IL-1 resulted in an increase in UMC adherence from 2.4% ± 0.6% to 6.5% ± 1.1% (mean ± SEM).

Histamine release from purified human UMC. To determine whether these purification procedures altered histamine releasability from human UMC, cells were challenged with polyclonal goat antihuman IgE or the calcium ionophore A23187 immediately after dispersement with collagenase (purity ≤1%) or after enrichment on Percoll gradients (purity ≥95%). As shown in Fig 3, the histamine release responses to anti-IgE or calcium ionophore A23187 were not altered as a result of purification procedures. In separate experiments, treatment of UMC with saturating and subsaturating concentrations of MoAb IV.3 (anti-CD32, FcyRII) did not induce histamine release or alter anti-IgE–induced release (n = 4, data not shown).

DISCUSSION

Methods for the isolation and purification of human mast cells from uterine tissues have permitted the phenotypic and functional analysis of this cell type for comparison with other previously isolated mast cells. Using immunofluorescence and flow cytometry, human UMC were found to express many surface antigens previously found on mast cells from other Some are relatively specific for the mast cell (IgE, the c-kit receptor recognized by MoAb YB5.B813-17) while others are expressed on leukocytes and other cells. Among the panel of surface antigens analyzed, any surface marker previously found on other mast cell types27 was also expressed on UMC. In contrast to other
mast cells, the β2 integrin p150,95 (CD11c/CD18) and one type of low-affinity receptor for IgG (FcγRII, CD32) were detected on human UMC and confirmed using two different monoclonals recognizing different epitopes on these molecules.\(^5\) With respect to the possible function of these surface molecules, data presented herein showed that treatment of human mast cells with IV.3 antibody (anti-CD32) failed to induce histamine release or alter anti-IgE-induced release. Although the exact functions of these structures on mast cells remain to be determined, the roles, on other cell types, of p150,95 (in cell adhesion) and FcγRII (in binding to aggregated IgG) are well documented.\(^5\) While the expression of these molecules on UMC provides evidence in support of mast cell heterogeneity, it is possible that purification techniques used for mast cells from other tissues, including the use of proteases, may alter specific epitopes recognized by the MoAbs. It is interesting to note, however, as has been suggested by Valent et al for other mast cells,\(^6\) that UMC display a number of surface antigens found on macrophages. This finding is true for CD11c/CD18, CD9, CD32, CD33, and others, and suggests a possible linkage between the lineage of mast cells and monocytes. The recent report that human skin mast cells may contain and release tumor necrosis factor, a cytokine produced by monocytes and macrophages, is also consistent with this concept.\(^7\)

The recent availability of MoAbs recognizing the β1 and β3 families of the integrin supergene family has now permitted analysis of expression of several of these surface antigens in immunophenotyping studies of mast cells. Expression of VLA-4 and VLA-5 α (CD49d and CD49e) and its common β subunit (CD29) was detected, as was expression of the α and β subunits of the vitronectin receptor (CD51/CD61). These results, along with the identification of p150,95 and absence of the selectin LECAM-1, recognized by MoAb Leu-8, are particularly intriguing in view of recent studies showing human mast cell migration in vivo, and chemotaxis and adhesion of mouse mast cell lines to laminin but not collagen or fibronectin in vitro.\(^8\)\(^-\)\(^10\) Although the expression of VLA-2, a receptor known to bind both collagen and laminin, and VLA-6 (the so-called laminin receptor) could not be detected on UMC, it is possible that binding to laminin occurs through other receptors such as VLA-1 or VLA-3 that were not analyzed in the present study.\(^11\) In addition, the fact that UMC bind to IL-1-activated endothelial cells and express p150,95, VLA-4, and VLA-5 suggests that these cells may interact with various ligands including an as yet unidentified cytokine-inducible counter-receptor for CD11c/CD18,\(^12\) the endothelial cell activation molecule VCAM-1 (recognized by VLA-4\(^\alpha\)), and the carboxy terminal connecting segment CS-1 region and arg-gly-asp (or RGD)-containing regions of fibronectin (recognized by VLA-4 and VLA-5, respectively).\(^13\)\(^,\)\(^14\) The vitronectin receptor (CD51/CD61), found on platelets, endothelium, tumor cells, and other cell types, is capable of binding to a variety of ligands in addition to vitronectin, including von Willebrand factor, fibrinogen, thrombospondin, and others.\(^15\) If in fact mast cells are capable of binding to specific extracellular matrix and endothelial cell proteins, this interaction may contribute to the localization of mast cells near vascular endothelium in many tissues. It is anticipated that further studies involving more extensive analysis of adherence responses will shed additional light on the function of human mast cell integrins and perhaps other adhesion molecules.

REFERENCES


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