In Vitro Functional Reactivities of Cutaneous Mast Cells From Patients With Mastocytosis

Michael D. Tharp, M.D.,* Basem Chaker, M.D., Marcia J. Glass, M.D., Rebecca Burton, B.S., and Leonard L. Seelig, Jr., Ph.D.†
Departments of Dermatology (MDT, BC, MJG, RB), Internal Medicine (MDT), and Cell Biology and Anatomy (LLS Jr), University of Texas Health Science Center at Dallas, Dallas, Texas, U.S.A.

Cutaneous mast cells from 3 patients with mastocytosis were evaluated for their morphologic characteristics and in vitro functional reactivities to different secretory agonists. By electron microscopy, mastocytosis mast cells appeared larger than normal skin mast cells, frequently had atypical, highly indented or bilobed nuclei, and each contained numerous, elongated cytoplasmic projections. Suspensions of mastocytosis mast cells were obtained from lesional skin biopsy specimens, and their response to both immunologic and nonimmunologic secretagogues was compared with mast cells from normal skin. Lesional skin mast cells had a net histamine release of 12.3% (± 1.3 SEM) and 31.1% (± 6.0 SEM) following stimulation with the purified human anaphylotoxin C3a and mouse monoclonal antihuman IgE antibodies, respectively. This specific release was similar to the responses observed in normal skin mast cells (11.5% ± 4.5 SEM and 16.7% ± 2.1 SEM, respectively). Mast cells from cutaneous lesions of mastocytosis also responded to the nonimmunologic secretagogues, morphine sulfate and calcium ionophore A23187 with a specific histamine release of 15.1% (± 1.2 SEM) and 39.8% (± 8.7 SEM), respectively. The results of this study demonstrate that mast cells from lesions of mastocytosis are morphologically atypical, but have a histamine content similar to normal skin mast cells and retain their functional reactivities to clinically relevant secretory stimuli. J Invest Dermatol 89:264–268, 1987

Mastocytosis represents a spectrum disorder resulting from an abnormal proliferation of tissue mast cells. The features of this disease process have been the subject of numerous reports [1–7]. In particular, several studies have focused on the atypical morphologic characteristics of mast cells in cutaneous lesions of mastocytosis at the ultrastructural level [8–12]. Because of this morphologic atypia, the functional reactivities of mast cells from patients with mastocytosis have been questioned [10]. Although the occurrence of both local cutaneous and systemic mast cell–mediated symptoms is well documented in patients with mastocytosis [2–4,13,14], we have been impressed that most of the mastocytosis patients under our care have few clinical complaints. This observation also has led us to question the responsiveness of mast cells from some patients with mastocytosis to different stimuli.

Our ongoing interest in human mast cell function has resulted in the development of a technique for obtaining viable, normal human cutaneous mast cells in suspension for in vitro functional studies [15,16]. In this report, we have applied this isolation method to lesional skin biopsy specimens from 3 patients with mastocytosis, and have examined the resulting mast cell suspensions for their in vitro responses to secretory agonists. In addition, we have assessed the morphologic characteristics of lesional skin mast cells from these 3 patients by electron microscopy and have compared these features with mast cells in normal skin. Our results confirm previous observations that mast cells in cutaneous lesions of mastocytosis are morphologically atypical by several parameters. However, despite these morphologic alterations, mastocytosis mast cells are similar to normal skin mast cells in their in vitro responses to different secretory stimuli.

MATERIALS AND METHODS

Reagents  Histamine dihydrochloride, N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), human serum albumin fraction V, porcine intestinal heparin, porcine kidney diaminopimelate oxidase (histaminase), gentamicin, hyaluronidase, deoxyribonuclease (DNase), and calcium ionophore A23187 (Sigma Chemical Co., St. Louis, Missouri); collagenase (Type III) (Worthington, Freehold, New Jersey); fetal calf serum (FCS), Alpha medium, L-glutamine, antibiotic-antimycotic mixture (GIBCO Laboratories, Grand Island, New York); morphine sulfate (Merck, Rahway, New Jersey); and [3H]S-adenosyl-methionine (76 Ci/mM) (Amersham, Arlington Heights, Illinois) were purchased from the sources cited. Mouse monoclonal antihuman IgE antibodies and purified human C3, were the generous gifts of Dr. J. Donald Capra, University of Texas Health Science Center, Dallas, Texas and Dr. Tony Hugli, Scripps Clinic and Research Foundation, La Jolla, California.

Preparation of Human Cutaneous Mast Cell Suspensions

Discarded human cutaneous tissue was obtained following circumcision of infants ranging in age from 3 days to 6 years. Following informed consent, shaved lesional skin specimens were
obtained after intradermal anesthesia with 1% lidocaine from 2 adult patients with the telangiectatic lesions of mastocytosis (patients 1 and 2) and a four-year-old child with papular lesions of mastocytosis (patient 3). All cutaneous specimens were placed immediately in Alphamedium, stored at 4°C and used within 2 h. A mixed suspension of dermal cells was prepared using a modified method as described previously [16]. Skin specimens were weighed and cut into 200-μm thick slices, washed twice in 15 ml of buffer (135 mM NaCl, 3.7 mM KCl, 5 mM Na3PO4, 1.5 mM CaCl2, 0.5 mM MgCl2, 25 mM HEPES, 5 mM glucose, heparin (7 μg/ml), pH 7.2), and added to 10 ml scintillation vials containing a buffer solution of collagenase (0.2 units/ml), hyaluronidase (0.2 units/ml), and DNase (0.5 mg/ml). Individual cells were harvested from tissue slices by passing the mixture over a fine mesh nylon screen. Cells were washed twice in 15 ml of ice cold buffer and resuspended for overnight culture in conditioned Alpha medium of 2 mM L-glutamine, 25 mM HEPES, gentamicin (10 μg/ml), antibiotic-antimycotic mixture (20 μl/ml), and 10% heat inactivated FCS. Passive sensitization of mast cells was accomplished by overnight incubation in conditioned Alpha medium with 10% human serum containing 4.4 mg/ml of IgE. Sample aliquots of mast cells recovered from overnight cultures were stained with crystal violet, conjugated avidin, and trypan blue. Human cutaneous mast cells were readily differentiated from other disaggregated dermal cells by their numerous cytoplasmic, metachromatic granules and their ability to bind conjugated avidin [15]. An average of 3.06 × 105 mast cells/mg of normal skin and 2.25 × 105 mastocytosis mast cells/mg of lesion tissue representing 13.2% (± 0.8 SEM) and 80.5% (± 5.0 SEM), respectively, of the total dermal cell population was routinely recovered. The mean mast cell viability from all specimens after overnight culture was 85.6% (± 1.9 SEM) as determined by trypan blue exclusion.

**Experimental Procedure for In Vitro Studies**

Approximately 3000 mast cells in 0.05 ml of buffer were incubated from 15–30 min at 37°C in a 12 × 100 mm polycarbonate tube with an equal volume of buffer alone, buffer containing calcium ionophore A23187 (3 μg/ml), C3 (1 μM) or morphenic sulfate (10 μM). Cells passively sensitized overnight were stimulated under the same conditions with mouse monoclonal antihuman IgE antibodies as previously described [16,17]. Each experimental condition was performed in triplicate. Mast cell stimulation was terminated by adding 0.4 ml of ice cold buffer to individual tubes that had been plunged in an ice water bath. The incubation supernatants were aspirated from the cell suspension following centrifugation (200 g) for 7 min at 4°C. The residual histamine in each sample was extracted by resuspending the cells in 1.0 ml of buffer and heating at 107°C in a heating block for 10 min. Following incubation with varying agonists, suspensions of mast cells were examined microscopically for their ability to exclude trypan blue (0.1%) and compared with buffer controls. No difference in mast cell viability (82.3% ± 3.0 SEM) was detected among the different experimental conditions.

**Histamine Assay**

Histamine released from cutaneous mast cells was measured by a modified single radioenzymatic method based on the work of Snyder et al [18]. Histamine N-methyltransferase was prepared from Sprague-Dawley rat kidneys using a modified purification scheme of Verberg et al [19]. Duplicate samples from each stimulated supernatant and from each residual cell pellet were assayed for histamine as previously described [16,17]. Standard curves for added authentic histamine (10–1000 pg) were determined for each assay. Correlation coefficients derived from linear regression analysis of the standard histamine curves ranged from 0.98–0.99. The presence of putative histamine in experimental samples was confirmed by degradation with porcine histaminase as described previously [17]. In all experiments the histamine in the supernatant and the residual cellular histamine in each sample were added to determine the total histamine present. The supernatant histamine was divided by the total histamine content in each sample to determine the percent histamine released during experimental conditions.

**Electron Microscopic Studies**

Representative samples of all tissue specimens were initially immersion-fixed in a solution containing 1% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer with 0.125 mM sucrose and 0.005 M CaCl2 (pH 7.2). Tissues were trimmed into small strips for orientation and fixed for 4 h at 4°C. Following a buffer rinse, specimens were postfixed in 1% osmium tetroxide buffered in 0.1 M cacodylate (pH 7.2) for 2 h. Fixed samples were dehydrated in a graded series of ethanol, followed by propylene oxide and embedded in epoxy plastic (Polys/Bed 812, Polysciences, Warrington, Pennsylvania). For microscopy, embedded specimens were oriented and glued on prepolymerized blocks so that sections were cut perpendicular to the upper dermal surface. For light microscopy, 1-μm sections were prepared on an LKB ultratome and stained with toluidine blue. Mast cell locations were identified in the dermis, and tissue blocks were trimmed for electron microscopy. Sections (40–60 nm) were cut with a diamond knife, mounted on colloidal-coated grids, stained with uranyl acetate and lead citrate, and viewed with a Joel 100 CX electron microscope. All dermal mast cells were photographed at 2000 ×, the prints were enlarged to 5600 ×, and the cells were assessed for their morphologic characteristics.

**RESULTS**

**Morphology of Mast Cells in Lesions of Mastocytosis**

Mast cell morphology in cutaneous lesions of mastocytosis and normal skin was assessed by electron microscopy. In normal cutaneous specimens these dermal cells appeared round to oval in shape, had slightly oval nuclei, and numerous cytoplasmic granules (Fig 1). In contrast, lesional skin mast cells from patient 1 appeared larger than normal, were irregular in overall shape, and frequently had bilobed nuclei (Fig 2). Numerous elongated cytoplasmic projections were evident in these cells. An apparent increase in the number and length of cytoplasmic extensions and granule size was observed in the lesional skin mast cells from patient 2, whereas in patient 3 large mast cells often had bilobed nuclei, and numerous atypical cytoplasmic projections (data not shown).

**In Vitro Functional Reactivities of Skin Mast Cells From Mastocytosis Patients**

Suspensions of lesional skin mast cells from each patient with mastocytosis were examined for their
histamine content and ability to respond in vitro to secretory agonists. The average mast cell histamine concentration among the 3 different patients ranged from 3.2 (± 0.1 SEM) pg to 4.2 (± 0.6 SEM) pg per cell, whereas a mean normal skin mast cell histamine content of 4.1 (± 0.8 SEM) pg was observed (Table I).

Using histamine release as an indicator of mast cell stimulation, the in vitro functional reactivities of mast cells from lesions of mastocytosis and normal skin were investigated. Cutaneous mast cell suspensions from each patient were incubated with the non-immunologic secretagogue, calcium ionophore A23187 (3 µg/ml), or buffer control for 15 min at 37°C, and their response was compared with normal skin mast cells. In all 3 patient mast cell preparations, significant histamine release above buffer controls was detectable, and in patients 2 and 3 this in vitro response was greater than normal skin mast cells (Table I). Suspensions of mast cells from patient 3 and from normal skin also were challenged with the secretory agonist, morphine sulfate (10 µM). Within 15 min, mastocytosis mast cells demonstrated a net histamine release of 15.1% (± 1.2 SEM) above buffer controls, whereas mast cells from normal skin specifically released 9.4% (± 1.1 SEM) of their total histamine content after morphine stimulation.

Mast cells from all 3 patients with mastocytosis also were investigated for their ability to respond to an immunologic stimulus. Due to limited cell numbers, lesional skin mast cells were challenged with either the purified human anaphylatoxin, C3a (1 µM), or with mouse monoclonal antihuman IgE antibodies. Mast cells from patients 1 and 2 released histamine (22.7% ± 1.3 SEM and 19.6% ± 1.3 SEM, respectively) in response to C3a stimulation. This reactivity was very similar to C3a-induced histamine release from normal skin mast cells (19% ± 4.5 SEM) (Table I). Mast cells from patient 3 were passively sensitized overnight with human IgE and examined for the presence of functional IgE receptors. Following challenge with anti-IgE antibodies, significant histamine release (41.2% ± 6.0 SEM) from these cells was observed (Table I).

**DISCUSSION**

Interest in lesional skin mast cells from patients with mastocytosis has resulted in a number of reports describing their ultrastructural morphology. In general, the most striking cell abnormality noted has been an increase in the number and length of cellular cytoplasmic projections [8-12]. Evaluation of the cutaneous mast cell infiltrates in our 3 patients with mastocytosis confirmed the presence of these atypical cell membrane changes. However, we also observed that these lesional skin mast cells varied from normal mast cells in their overall size and nuclear configuration. Because of these atypical morphologic features, we questioned whether lesional mast cells from our patients with mastocytosis would respond normally in vitro to different secretory agonists.

Using a technique developed in our laboratory, we obtained suspensions of mast cells from lesions of mastocytosis with a purity of ≥ 80%. When compared with normal skin specimens on a milligram per wet weight basis, lesions of mastocytosis yielded approximately 7 times more mast cells using this isolation procedure. It is of interest that we have observed similar increases in mast cell numbers above normal controls in histologic sections of the telangiectatic macules of mastocytosis [20].

The in vitro functional reactivities of mastocytosis mast cells to 2 different immunologic stimuli were examined. Despite abnormal cell surface morphology, mastocytosis mast cells demonstrated functional receptors for the anaphylatoxin C3a, and for IgE antibodies, and in general, the magnitude of these functional responses was at least equal to normal skin mast cells. Both IgE antibodies and C3a, are known to be important in vivo immunologic agonists for the tissue mast cell [16,21,22], and our experiments indicate that mast cells in lesions of mastocytosis are capable of responding to these stimulatory signals.

**Table I. In Vitro Stimulation of Mast Cells From Lesions of Mastocytosis**

<table>
<thead>
<tr>
<th>Skin Mast Cell Source</th>
<th>Histamine Content Per Mast Cell (pg)</th>
<th>% Histamine Release (± SEM)ᵃ Stimulus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Buffer</td>
</tr>
<tr>
<td>Mastocytosis Patient 1</td>
<td>4.2 (± 0.6)</td>
<td>8.1 (± 1.2)</td>
</tr>
<tr>
<td>Mastocytosis Patient 2</td>
<td>3.2 (± 0.1)</td>
<td>9.7 (± 4.3)</td>
</tr>
<tr>
<td>Mastocytosis Patient 3</td>
<td>3.3 (± 0.5)</td>
<td>12.8 (± 0.8)</td>
</tr>
<tr>
<td>Mean</td>
<td>3.6 (± 0.3)</td>
<td>10.1 (± 1.7)</td>
</tr>
<tr>
<td>Normal skinᵇ</td>
<td>4.1 (± 0.8)</td>
<td>7.5 (± 2.0)</td>
</tr>
</tbody>
</table>

ᵃn = 3 for each experimental condition.
ᵇND: not done.
ᶜStimulation with mouse monoclonal antihuman IgE antibodies after overnight sensitization with human IgE (4.4 mg/ml).
ᵈn = 9 for each experimental condition.
Our studies also showed that mastocytosis mast cells released histamine following stimulation with the nonimmunologic secretagogues, morphine sulfate and calcium ionophore A23187. Morphine has been shown to induce in vitro histamine release from normal skin mast cells but not human peripheral blood basophils [16,23,24]. In addition, this opiate has been reported to provoke episodes of cutaneous flushing and hypotension in patients following its parenteral administration as an anesthetic agent. A central role for mast cell-derived histamine release in these reactions has been strongly suggested by the observation that pretreatment of patients with combined histamine; (H₁) and histamine; (H₂) receptor antagonists significantly reduces the hemodynamic changes induced by morphine [25,26]. Because cutaneous mastocytosis mast cells release histamine following exposure to morphine in vitro, the in vivo use of this agent and related compounds in patients with mastocytosis, could potentially induce systemic symptoms resulting from mast cell degranulation.

Calcium ionophore A23187 also is recognized as a mast cell secretagogue the effect of which is mediated primarily by facilitating the transportation of calcium into the cell [27,28]. Ultrastructural studies have demonstrated that the morphologic changes that occur during A23187-induced mast cell degranulation are very similar to those observed at the time of anaphylactic mediator release [29,30]. In our experiments, 2 of the 3 patient mast cell preparations showed an apparent augmented response to A23187 when compared with normal controls. It is conceivable that skin mast cells from some patients with mastocytosis may have a defect in the regulation of mediator release during a time of marked intracellular calcium influxes. However, the in vivo relevance of this in vitro observation remains uncertain since neither patient 2 nor patient 3 experienced significant cutaneous or systemic mast cell-related symptoms.

Recent reports have indicated that suspensions of canine mast cells recovered from subcutaneous mastocytomas release mediators after in vitro stimulation with immunologic and nonimmunologic agonists [31,32]; however, the magnitude of these responses has not been correlated with normal dog skin mast cells. In this study we have demonstrated that suspensions of human mast cells can be obtained from papular and telangiectatic macular lesions of mastocytosis as well as normal skin for in vitro functional studies. In contrast to the well-differentiated dog mastocytosis mast cells, lesional skin mast cells from our patients demonstrated several atypical morphologic features, yet, were responsive to both immunologic and nonimmunologic stimuli. Thus, we conclude from this study that despite their morphologic atypia, lesional skin mast cells from our 3 mastocytosis patients had histamine content very similar to normal cutaneous mast cells and retained their functional reactivities to clinically relevant secretory agonists.

The authors wish to thank Mrs. Teresa Polack and Mr. Charles Lynch for their expert technical assistance and Ms. Diane Dracopoulos for her fine preparation of the manuscript.

REFERENCES

VOL. 89, NO. 3 SEPTEMBER 1987

M AST CELL FUNCTION IN MASTOCYTOSIS

