

PURIFICATION AND CHARACTERIZATION OF HUMAN SKIN MAST CELLS

Evidence for Human Mast Cell Heterogeneity¹

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Our previous studies of human lung and intestinal mast cells failed to show the heterogeneity found among mast cells in murine species. Recently, we and others have developed techniques for the enzymatic dispersion of human neonatal skin mast cells. In addition, we are now able to make single cell suspensions of mast cells from adult skin and to purify these cells to near homogeneity. Comparative studies of mast cells from these several sources have uncovered several major differences among them.

Adult and neonatal skin mast cells themselves differ in that the former are 10-fold less sensitive to goat anti-human IgE, with maximal release occurring at 3.0 and 0.3 $\mu\text{g/ml}$, respectively. Skin mast cells also differ in optimal temperature for release: adult mast cells respond maximally at 23 to 30°C and neonatal cells at 37°C.

Skin mast cells from both sources are dramatically different from lung and intestinal mast cells in two aspects. First, skin mast cells are quite responsive to several stimuli—morphine sulfate (10^{-4} to 10^{-6} M), substance P (10^{-5} to 10^{-7} M), compound 48/80 (10 to 0.1 $\mu\text{g/ml}$), f-Met peptide (10^{-6} M), and C5a (10^{-8} M)—to which the other mast cells fail to respond. Second, although stimulated skin mast cells produce prostaglandin D₂, little leukotriene C₄, if any, is generated, unlike lung or intestinal mast cells. These differences in inflammatory potential among human mast cells from various sites have important implications for the management of allergic and inflammatory responses.

The concept of mast cell heterogeneity has been studied extensively over the past 20 years, although much of the data supporting this hypothesis has been derived from

work on the murine species (1–5). We have previously examined the question of mast cell heterogeneity in humans by using suspensions of mast cells obtained from lung parenchyma and bowel mucosa. These cells were found to be morphologically similar and we were unable to detect any functional differences with regard to the mediators released or their response to stimuli (6–8), thus casting some doubt on the possibility of mast cell heterogeneity in man. However, recent work with the use of immunochemical staining has demonstrated differences between human mast cells derived from the lung and mucosa of the small intestine and those of the skin and submucosa of the large bowel (9). In addition, it has been shown that human skin slices release histamine after contact with several stimuli that do not elicit a response from human lung mast cells (10–13).

Techniques to enzymatically disperse skin mast cells from neonatal foreskin have recently been developed both in this laboratory and elsewhere (10–14). We have now developed a method for the enzymatic dispersion of mast cells from adult skin obtained from mastectomies or cosmetic surgery procedures and have been able to purify these dispersed skin mast cells to greater than 85% homogeneity. Thus, we have been able to characterize the responses of skin mast cells to different stimuli while minimizing the effects of contaminating cells. We have also compared human blood basophils with mast cells derived from lung, bowel, and the skin for evidence of heterogeneity, whereas, most previous studies have relied on comparisons from the literature.

In this report, we explore the characteristics of neonatal and adult skin mast cells and present evidence of functional heterogeneity of human mast cells derived from different anatomic sites as well as between human mast cells from adult and neonatal sources. One of the more interesting aspects of this heterogeneity is that human skin mast cells respond to a variety of stimuli that are ineffective in lung and gut mast cells. Furthermore, these cells have a very limited ability to metabolize arachidonic acid to leukotriene C₄(LTC₄)⁴ in contrast to human lung and bowel mast cells (6–15).

⁴ Abbreviations used in this paper: LTC₄, leukotriene C₄; CMF-HBSS, calcium and magnesium free Hanks' balanced salt solution; anti-IgE, goat anti-human IgE; FMLP, *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine; PGD₂, prostaglandin D₂; HPLC, high pressure liquid chromatography; PIPES, piperazine-*N,N'*-bis[2-ethane-sulfonic acid]; PAG, a buffer consisting of 25 mM PIPES, 110 mM NaCl, 5 mM KCl, 0.1% dextrose, and 0.003% human serum albumin; PAGCM, PAG supplemented with 1 mM CaCl₂ and 1 mM MgCl₂.

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MATERIALS AND METHODS

Reagents. The following were purchased: piperazine-*N,N'*-bis[2-ethane-sulfonic acid] (PIPES), penicillin, elastase, and hyaluronidase (Sigma, St. Louis, MO); Hanks' balanced salt solution (HBSS), heat-inactivated newborn calf serum, collagenase, and fetal calf serum (FCS) (GIBCO, Grand Island, NY); DNase and calcium ionophore A23187 (Calbiochem, San Diego, CA); RPMI 1640 medium with 25 mM HEPES, Eagle's minimum essential medium (MEM) and gentamicin (Whitaker, M.A. Bioproducts, Walkersville, MD); Percoll (Pharmacia Fine Chemicals, Piscataway, NJ); substance P (Peninsula, Belmont, CA); and *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP) (Vega Biochemicals, Tucson, AZ). C5a was generously provided by Dr. K. B. Yancey, USUHS, Bethesda, MD. The goat anti-human IgE (anti-IgE) utilized has been described (16).

Buffers. The following buffers were prepared: PAG consisted of 25 mM PIPES, 110 mM NaCl, 6 mM KCl, 0.1% dextrose, and 0.003% human serum albumin. Mediator release experiments were carried out in PAG supplemented with 1 mM CaCl₂ and 1 mM MgCl₂ (PAGCM).

Isolation of neonatal skin mast cells. Human neonatal skin was obtained from elective circumcisions of 3- to 5-day-old infants. The tissue was immediately placed in Eagles' MEM at 4°C and used within 12 hr. It was chopped into 1- to 2-mm fragments and washed twice in calcium- and magnesium-free HBSS (CMF-HBSS) at 23°C. Tissue fragments were then incubated in HBSS with 4 mg/ml collagenase, 1 mg/ml hyaluronidase, and 1000 U/ml DNase in a total volume of 1 ml/g wet weight of tissue for 3 hr, at 37°C with constant stirring. The digested tissue was filtered over Nitex cloth (150-μm pore size; Tetko, Elmsford, NY), the resultant cell suspension was washed three times in CMF-HBSS and the mast cells counted on a Spiers-Levy eosinophil counter after staining with Alcian blue at pH 1.0.

Short term culture. The cells (2×10^5 mast cells/ml) were suspended in RPMI 1640 with 25 mM HEPES, 1% penicillin, 1% gentamicin, 5% FCS, and 10% human serum containing 6000 ng of IgE/ml of serum. The suspension was inoculated into a 25-cm² tissue culture flask and incubated overnight at 37°C in humidified 95% air, 5% CO₂. After 12 hr of culture the cells were washed twice in PAG at 23°C. The mast cells were recounted by using Alcian blue stain and the viability was assessed by erythrosin B exclusion.

Isolation of adult skin mast cells. Adult skin was obtained from either mastectomies for breast cancer or from elective cosmetic surgery procedures. Tissue was placed in Eagle's MEM at 4°C immediately and used within 1 hr. The skin was separated from the s.c. fat by blunt dissection and full thickness punch biopsies were taken by using a 4-mm Baker's biopsy punch (Key Pharmaceuticals, Miami, FL). Biopsies were further chopped to a size of 1 mm and washed once in CMF-HBSS at 4°C to remove excess fat. The fragments were washed twice in CMF-HBSS at 23°C and incubated in a solution of CMF-HBSS containing 20 mg collagenase/g wet weight of tissue, 5 mg hyaluronidase/g wet weight, and 1000 U/ml DNase for 3 hr at 37°C with constant stirring. The isolated cells were separated from the partially digested tissue fragments by filtration through Nitex cloth (150-μm pore size) and reserved. The remaining tissue was subjected to an additional digestion with the use of a fresh solution of the enzymes for a further 2 hr at 37°C. The cells were separated from the tissue fragments by filtration through Nitex cloth, washed twice, combined with the cells from the first digestion and washed again in CMF-HBSS. Mast cells were stained with Alcian blue at pH 1.0 and counted in a Spiers-Levy eosinophil counter.

Short term culture of adult skin mast cells. Cells obtained from the two-step digestion were resuspended in RPMI 1640 containing 25 mM HEPES, 2 mM L-glutamine, 1% penicillin, 1% gentamicin, and 10% FCS. The suspension was then inoculated in 5-ml aliquots (2×10^5 mast cells/ml) into 25-cm² tissue culture flasks and incubated in humidified 95% air, 5% CO₂ at 37°C. Mast cells were harvested after 12 hr, washed in PAG twice at 23°C, counted by using Alcian blue at pH 1.0, and stained with erythrosin B to determine viability. For the passive sensitization experiments, cells were incubated overnight in the above mixture with the addition of 10% human serum containing 6000 ng of IgE/ml of serum.

Purification of skin mast cells. Isotonic Percoll was prepared by mixing nine parts Percoll and one part 250 mM PIPES buffer and further diluted with 25 mM PIPES to give final concentrations of 80% and 50% Percoll in PIPES buffer. Gradients were prepared in 50-ml polypropylene centrifuge tubes by layering 10 ml of 50% Percoll over a cushion of 10 ml 80% Percoll. Cells were washed in PAG, resuspended to a concentration of 2×10^6 mast cells/10 ml in PAG buffer, layered over the 50% layer of Percoll, and centrifuged at $300 \times G$ at 23°C for 15 min. The supernatant, interface, and pellet were collected and the number of mast cells in each fraction was estimated after Alcian blue staining.

Preparation of lung mast cells. Lung mast cells were obtained by using the methods previously described (17, 18).

Preparation of bowel mast cells. Single cell suspensions of bowel mast cells were prepared by using the technique of Fox et al. (6).

Basophil preparation. For the arachidonic acid experiments, whole blood was anticoagulated with EDTA, diluted with saline, and layered over a cushion of isotonic Percoll (sp. g., 1.079). The tubes were spun at $300 \times G$ for 15 min and the mononuclear cells recovered from the plasma-Percoll interface. The cells were washed once in EDTA-saline, once in PAG-EDTA, twice in PAG, resuspended in the appropriate volume of PAGCM, and challenged. For all other experiments, basophils were prepared by using the method previously described (19).

Measurement of histamine content of whole skin. Samples of chopped skin were weighed, 1-g portions were boiled, and the amount of histamine released into the supernatant was measured by using a fluorometric assay for histamine (20).

Histamine release assay. Cells were suspended in PAGCM at a concentration of 2×10^4 mast cells/experimental tube and challenged in duplicate with anti-IgE, A23187, morphine sulfate, substance P, C5a, or f-Met peptide in a volume of 0.1 ml for 30 min at 37°C. For the experiments to establish the kinetics, calcium, and temperature dependence of the release process, conditions were adjusted accordingly. The concentration of anti-IgE was chosen to give maximum histamine release. Supernatants were assayed for histamine by the automated fluorometric technique of Siraganian (20). Results were expressed as the percentage of total histamine content, determined by lysis of cells in 2% perchloric acid, and corrected for spontaneous histamine release as determined from buffer control tubes.

Measurement of arachidonic acid metabolites. Cells were resuspended in PAGCM at a concentration of 2×10^5 cells/ml and challenged with the optimum dose of anti-IgE or A23187. The LTC₄ and PGD₂ produced was measured by using radioimmunoassays previously described (21, 22). For PGD₂ assays cellfree supernatants were analyzed within 24 hr of challenge to minimize degradation of the compound. For degradation studies, cellfree supernatants were stored in buffer at -20°C and samples were analyzed for PGD₂ immediately and at 1- and 2-wk intervals. In two experiments the prostanooids released from skin mast cell preparations were measured by negative ion mass spectrophotometric analysis (23). To assess the ability of the crude cells to metabolize exogenous LTC₄, 2×10^5 /ml mast cells were incubated with ³H-labeled LTC₄ and the cells challenged with either anti-IgE or A23187. Samples were taken at the specified time intervals and immediately centrifuged to remove the cell pellet. The resulting supernatants were extracted with chloroform/ethanol (24) and analyzed by high pressure liquid chromatography (HPLC) (25). The organic solvents were removed under reduced pressure, a scintillation mixture was added to the residue, and the profile was compared with elution profiles of authentic nonlabeled standards.

RESULTS

Average mast cell yield and purity. Neonatal foreskins yielded an average of $8 \pm 2 \times 10^5$ mast cells/g of skin with a purity of 10%; the remaining cells were largely endothelial cells with some fibroblasts. This represented the recovery of 30 to 40% of the total histamine content of neonatal skin. In the adult skin the mast cell yield was lower, averaging $5 \pm 0.5 \times 10^5$ mast cells/g of tissue or 20 to 30% of the histamine found in adult skin. Again purity was approximately 10%. The average histamine content of mast cells from both neonatal and adult skin was 3.5 ± 0.5 pg/cell.

Separation of crude preparations of adult skin mast cells on Percoll density gradients demonstrated that the majority of the mast cells were located at the interface between the 50 and 80% layers at an average purity of $92 \pm 7\%$ (Table I). The remainder of the cells at the interface were endothelial cells. Mast cells recovered from the upper layer were dead as assessed by erythrosin B uptake. The majority of cells contained in the pellet were endothelial cells and fibroblasts. Approximately 45 to 60% of mast cells that were purified initially on the Percoll gradient were recovered.

TABLE I
Percoll purification of human skin mast cells^a

Location	Mast Cells	Endothelial	Fibroblasts	Others
50% interface	11 ± 1	75 ± 2	10 ± 1	5 ± 1
80% interface	92 ± 7	6 ± 5	2 ± 1	<1
Pellet	10 ± 2	9 ± 1	35 ± 5	46 ± 4

^a Data expressed as percentage of total cells at each interface ± SEM.

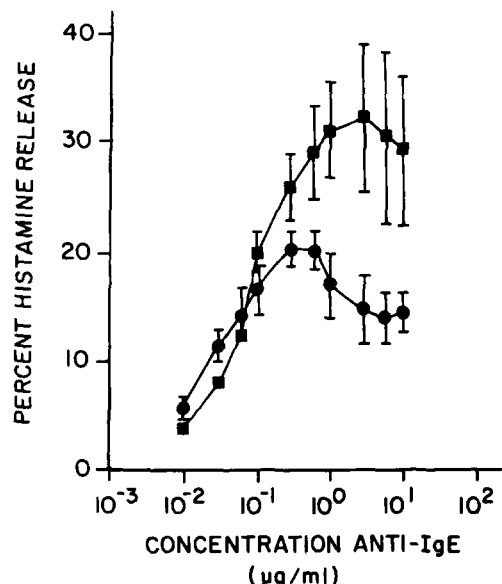


Figure 1. Dose dependence of anti-IgE-induced histamine release from human neonatal (●) and adult (■) skin mast cells. All experiments were carried out at 37°C with 1 mM CaCl₂. (The mean of six experiments ± SEM is shown.)

Parameters of histamine release. Both passively sensitized neonatal mast cells and nonsensitized adult skin mast cells responded to challenge with anti-IgE (Fig. 1). The neonatal cells demonstrated maximal release at a concentration of 0.3 μg/ml of anti-IgE, whereas the adult cells required a 10-fold higher concentration. This difference appears to be an intrinsic property of these cells because passive sensitization of mast cells from adult skin did not change their response to anti-IgE. Both cell types demonstrated the excess stimulus inhibition observed in other mast cells. In the neonatal skin mast cells, however, a 10-fold increase above the maximal dose of anti-IgE resulted in a 30% inhibition of release whereas a similar increase caused only 8% inhibition in the adult cells. The concentration of anti-IgE required for maximal histamine release from basophils is 0.1 μg/ml whereas the concentration required for maximal release in lung and bowel mast cells is 3 μg/ml (6, 17, 18). Thus, not only did neonatal skin mast cells differ from adult skin mast cells in their responsiveness to anti-IgE, they differed from other mast cells as well. Both neonatal and adult skin mast cells displayed similar kinetics, reaching maximal release after 4 min. Lung and bowel mast cells release mediators at the same rate whereas in basophils maximal release is achieved more slowly (data not shown) (17, 26).

Histamine release from adult skin mast cells also differed from that observed in mast cells from other tissue locations (Fig. 2). The maximal release occurred at 30°C, with approximately the same amount of release at 23°C and 37°C (~75% of maximal release). The mast cells from neonatal skin, however, behaved like mast cells from

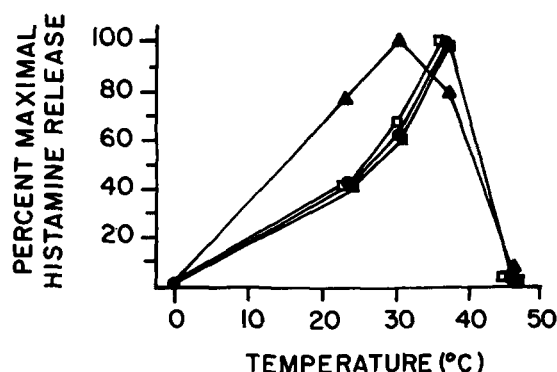


Figure 2. Temperature dependence of anti-IgE-induced histamine release from human mast cells: adult skin (▲), neonatal skin (●), lung (■), and intestinal (□). Adult, lung, and intestinal mast cells were challenged with 3 μg/ml of anti-IgE, neonatal skin challenge with 0.3 μg/ml. (Data are mean of six experiments.)

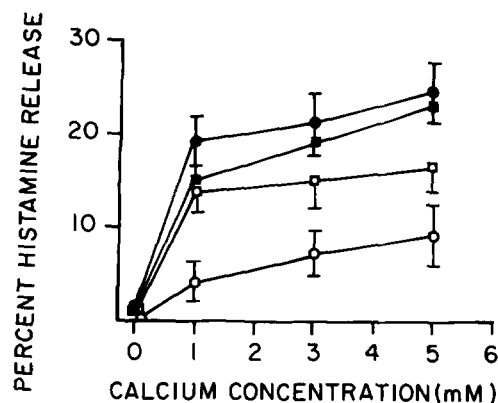


Figure 3. Calcium dependence of anti-IgE-induced histamine release at four concentrations of anti-IgE: 10 μg/ml (□), 1 μg/ml (●), 0.1 μg/ml (■), and 0.01 μg/ml (○). All experiments carried out at 37°C for 30 min. (Data are the mean of three experiments ± SEM.)

other organs, showing maximal release at 37°C which fell to well under half maximal at 23°C. This suggested that the change in optimum temperature was a function of age. In a single experiment with skin mast cells from an elective circumcision done on a 6-month-old boy, the adult pattern of temperature dependence was already present. We have observed that the anti-IgE dose-response curve shifts as a function of temperature in the basophil, so we examined this parameter in adult skin mast cells. Although there was, of course, a difference in the amount of histamine release at each concentration of anti-IgE, the shape of the curve and the concentration yielding maximal release did not change as the temperature was varied (data not shown).

Tharp et al. reported that histamine release from human skin mast cells occurred over a very narrow calcium concentration range, with maximum release at 3 mM and a dramatic decrease at 1 or 5 mM (14). In our hands, skin mast cells failed to release in the absence of calcium, 1 mM Ca²⁺ was found to be essentially optimal with no significant increase in histamine release occurring as the Ca²⁺ concentration was increased to 5 mM (Fig. 3). The same relationships were obtained at several different anti-IgE concentrations, and we found no difference between neonatal and adult mast cells.

Non-IgE-mediated histamine release. Compared with basophils, mast cells from the lung and bowel responded to few stimuli. Skin mast cells were more reactive. As previously reported (27), morphine caused release from

neonatal cells; it was also active in adult skin mast cells (Fig. 4A) (27). In simultaneous experiments, this drug failed to cause histamine release from either lung or bowel mast cells or from basophils. Morphine-induced release was both temperature- and calcium-dependent; no release was observed in the absence of calcium or at 0°C.

Substance P is also an active secretagogue for neonatal and adult skin mast cells, whereas lung and bowel mast

cells and basophils do not respond to this stimulus (Fig. 4B). As with morphine, this release was temperature- and calcium-dependent. In a similar manner, the classical murine mast cell secretagogue, compound 48/80, does not cause histamine release from human basophils or from the mast cells studied to date, but is active on both adult and neonatal mast cells (Table II). Of possibly more biologic significance, the skin mast cells, like basophils, responded to C5a, even though this stimulus failed to stimulate lung mast cells. A similar set of observations was made with the putative bacterial secretagogue, FMLP. The skin mast cells and basophils responded whereas the lung mast cells did not. It is interesting to note that the intradermal injection of these secretagogues caused a wheal and flare reaction in the skin of all patients, regardless of their allergic status (28-33). As expected, the calcium ionophore A23187 caused substantial levels of histamine release from all cell types.

Arachidonic acid metabolism. Perhaps the most interesting difference between skin mast cells and their counterparts in other tissues relates to arachidonic acid metabolism. Our expectation, based on previous studies with lung mast cells, was that the skin cells would produce significant quantities of both LTC₄ and PGD₂ (6, 34). When early studies indicated that LTC₄ was not released in conjunction with histamine after challenge with either anti-IgE or the calcium ionophore, we adopted a protocol in which control cells (i.e., lung mast cells or basophils) were compared in the same experiments and assays as the skin cells to provide a positive control. These results are summarized in Figure 5. In four experiments with adult and four with neonatal skin mast cells, LTC₄ could be detected at twice the limit of assay sensitivity of 2.5 ± 0.3 ng/10⁶ ml cells in only two of the eight instances (once in adult skin and once in neonatal skin). The quantities produced in the positive experiments were too small (average, 3.5 ng/10⁶) to permit HPLC analysis to confirm the presence of LTC₄. In neither of the positive experiments did skin mast cells generate as much as 5% of the LTC₄ made by control cells. Our failure to detect LTC₄ in the majority of our experiments and the small quantities found in the remainder was not caused by the rapid metabolism of LTC₄ or LTE₄ that occurs in the bowel mast cells. Skin mast cells (2×10^6 cells/ml) of 20 to 30% purity were incubated with 10⁻⁷ M of exogenous [³H]LTC₄ for 60 min at 37°C in the presence and absence of 3 µg/ml anti-IgE. HPLC analysis revealed that less than 5% of the [³H]LTC₄ was converted to LTD₄ or LTE₄ under

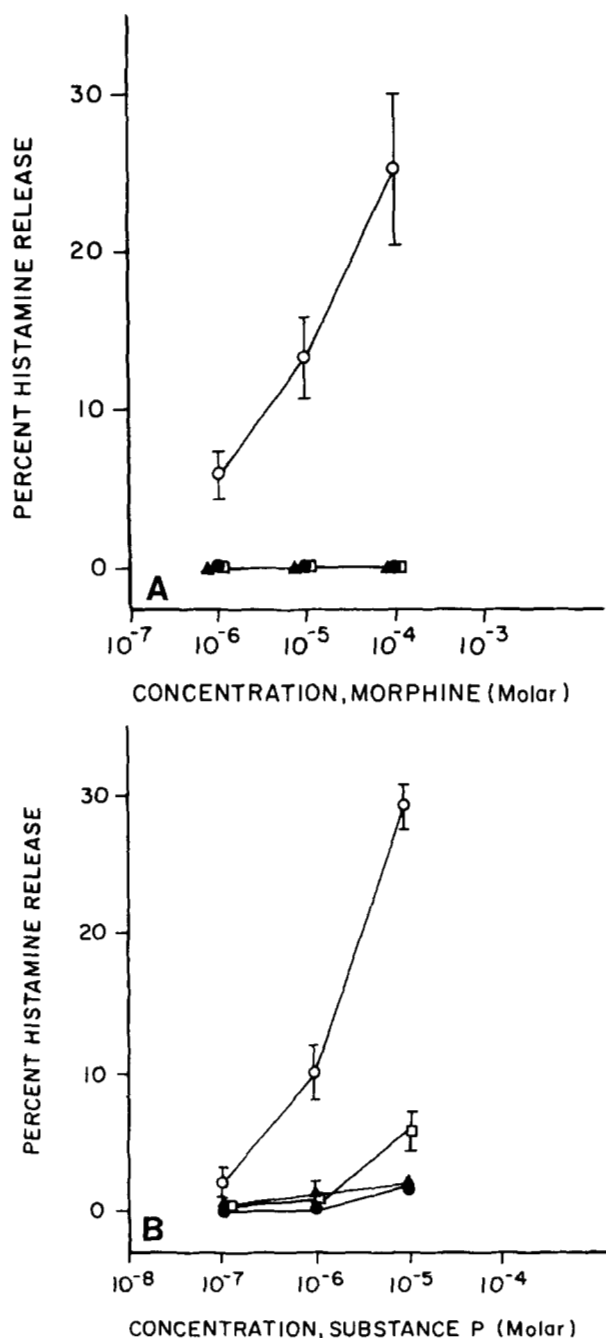


Figure 4. A. Dose dependence of morphine sulfate-induced release from human histamine-containing cells: skin mast cells (O), lung mast cell (▲), intestinal mast cells (□), and basophils (●). All experiments were carried out at 37°C, 1 mM CaCl₂ for 30 min. (Data are the mean of six experiments $\pm$ SEM.) B. Dose dependence of substance-P-induced histamine release from human histamine-containing cells: skin mast cells (O), lung mast cells (▲), intestinal mast cells (□), and basophils (●). All experiments were carried out at 37°C with 1 mM CaCl₂ for 30 min. (Data are the mean of six experiments $\pm$ SEM.)

Stimulus	Adult SMC ^b	Neonatal SMC	Lung MC	Basophils
48/80				
10 µg/ml	27 $\pm$ 3 ^c	29 $\pm$ 3	0	0
1 µg/ml	15 $\pm$ 3	8 $\pm$ 2	0	0
0.1 µg/ml	2 $\pm$ 1	0	0	0
A23187				
1 µg/ml	44 $\pm$ 4	43 $\pm$ 4	60 $\pm$ 5	62 $\pm$ 2
F-met				
1 µM	10 $\pm$ 7	10 $\pm$ 10	0	30 $\pm$ 3
C5a				
10 nM	14 $\pm$ 3	12 $\pm$ 2	0	30 $\pm$ 4

^a Results are percentage of total histamine released. For the mast cell experiments, n = 3 and for the basophil experiments, n = 9. All values are expressed as mean $\pm$ SE.

^b SMC, skin mast cells; MC, mast cells.

^c % Histamine release.

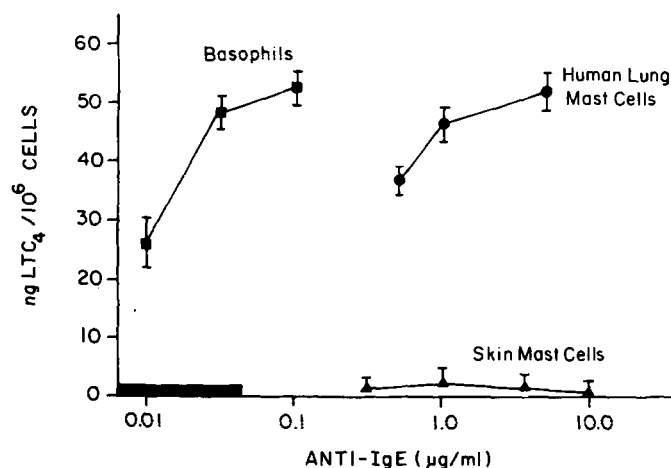


Figure 5. LTC₄ production from human histamine-containing cells after stimulation with anti-IgE (concentration $\mu\text{g/ml}$ on x-axis): basophils (■), lung mast cells (●), and skin mast cells (▲). The limit of sensitivity of the radioimmunoassay is indicated by the black bar. (Data are the mean of four experiments $\pm$ SEM.)

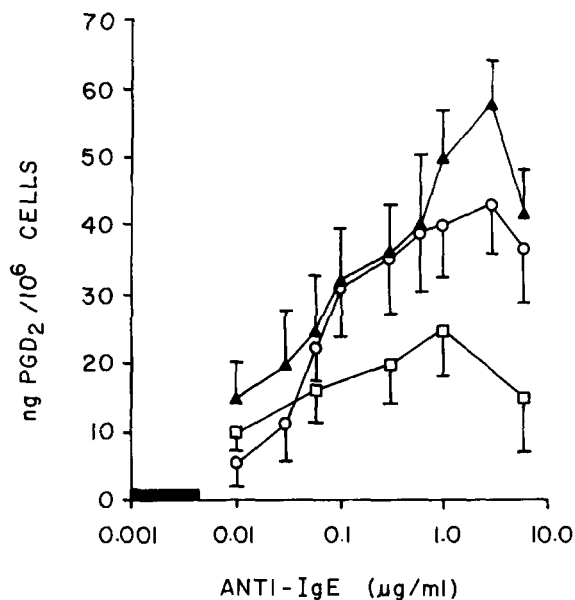


Figure 6. PGD₂ production by human mast cells after stimulation with anti-IgE: lung mast cells (▲), skin mast cells (○), and intestinal mast cells (□). Limit of detection for the assay is indicated by the black bar. (Data are mean of six experiments $\pm$ SEM.)

either condition. We conclude from these data that skin mast cells probably do generate LTC₄ but to a much lesser extent than lung or gut mast cells or basophils.

In contrast to the data on LTC₄, the cyclo-oxygenase product, PGD₂, appears to be the primary metabolite of arachidonic acid made by the skin mast cell. After stimulation with anti-IgE, skin mast cells produce PGD₂ in quantities similar to those of lung and bowel mast cells ($43 \pm 8.5 \text{ pg}/10^6 \text{ cells}$) (Fig. 6). These findings were confirmed by gas capillary, negative ion mass spectrophotometry. This finding differs from our previous report that PGD₂ was not generated by skin mast cells (35). This discrepancy is explained by the rapid degradation of the PGD₂ from skin mast cell preparations over short periods of time. As shown in Table III, more than 90% of existing PGD₂ is degraded during storage of cellfree supernatants at -20°C in 7 days. Of interest is the fact that in preparations in which the percentage of mast cells was increased (and therefore the number of contaminating cells

TABLE III
Destruction of PGD₂ in cellfree supernatants derived from skin mast cells as a function of time

Mast Cell Purity	PGD ₂ (pg/0.1 ml)		% Destruction
	Assayed immediately	Assayed after 7 days	
Expt. 1, 6%	364	32	91
Expt. 2, 7%	333	52	85
Expt. 3, 78%	259	50	81
Expt. 4, 85%	548	148	73

was decreased), there was apparently less destruction of PGD₂ suggesting that contaminating cells may play a role in this phenomenon. It is of note that this marked degradation of PGD₂ is not seen in lung mast cell preparations, suggesting that enzymes or cells not found in the lung preparations may be active in the skin.

DISCUSSION

Because of the relatively clear-cut findings which indicate that there are at least two types of murine mast cells, there has been a great deal of interest in whether such heterogeneity occurs in human mast cells (1-3). The difference in murine cells was first noted by Enerbäck, based on differences in fixation and staining characteristics and has been extended by the work of several investigators who demonstrated that mast cells from different anatomic locations produce different mediators, respond to different secretagogues, and are affected differently by pharmacologic agonists (2-5, 27, 30-33). The most recent work by Kitamura indicates that these differences can be induced and that mast cells can be changed from one type to another, depending on the local environment (36).

Our own studies initially suggested that this heterogeneity did not translate to man. The so-called "connective tissue" mast cells from the human lung and the "mucosal" mast cells from the human gastrointestinal tract were identical in terms of response to secretagogues, types of mediators released, and susceptibility to inhibition by different pharmacologic agonists (6). It was clear, however, that these cells were different in these respects from the basophil, the histamine-containing, IgE receptor-bearing cell found in the blood (37). The present manuscript extends these studies to mast cells from a third organ, the skin. The function of skin mast cells has been studied for the last 15 or 20 yr but, until very recently, the studies involved chopped skin, a system that was so poorly reproducible and so lacking in flexibility that only simple questions could be addressed. More recently, several groups have initiated studies with dispersed mast cells by using neonatal skin obtained by circumcision (10, 14). This present study extends their work by developing techniques to study adult skin, and by describing a simple technique that leads to rapid purification of these cells to near homogeneity. It is worth noting that the technology required to produce dispersed skin mast cells is simpler than that required in the human lung or gut. To disperse these latter cells, powerful proteolytic enzymes were necessary, whereas, in the present procedure, these enzymes are not required. Because in our previous work we have demonstrated that the harshly treated mast cells are not substantially different from those obtained by mechanical means, we can expect that the present preparation reflects the function

of the skin cells *in vivo* (38).

Before turning to differences between mast cells derived from the skin and those contained in other organs, there are two interesting differences between cells obtained from neonatal vs adult tissue. The first concerns sensitivity to stimulation by IgE aggregation. The adult skin mast cells show optimal release with 3 $\mu\text{g}/\text{ml}$ of anti-IgE, precisely the same concentration that has been described for lung and gut mast cells, whereas the human basophil is approximately 30 times more sensitive (16, 17). The neonatal mast cell resembles the basophil in being 10-fold more sensitive than the adult cell. The most obvious explanation for this difference, *i.e.*, that it reflects the passive sensitization of the neonatal but not the adult cells, does not seem adequate because passive sensitization of the adult cells does not increase sensitivity. Moreover, it does not seem to reflect any general increase in reactivity because the neonatal mast cells are no more sensitive to the other secretagogues we have studied. Indeed, if one compares the basophils of young and aged individuals, the cells from older individuals are more sensitive to anti-IgE than are those from younger individuals (39).

Another interesting difference between neonatal and adult skin mast cells was that the neonatal cells displayed maximal release at 37°C with reduced release at 30°C and only fractional release at 23°C. Release from the adult mast cells, on the other hand, peaked at 30°C and a substantial percentage of the histamine was released at 23°C. It seems likely that this is an acquired characteristic, because the neonatal cells have been in a 37°C environment whereas adult skin temperature is considerably lower. This hypothesis was supported by the single experiment indicating that skin from a circumcision performed at 6 mo behaved like adult skin.

The first major difference, then, between skin mast cells and those of other organs was the temperature sensitivity referred to above. Mast cells from other organs have a temperature optimum of 37°C rather than 30°C and release less than one-third of their histamine at 23°C. However, this was only one of several striking differences between cutaneous mast cells and those obtained from lung or gut. Although cutaneous mast cells were found to be similar to other mast cells in the fundamentals relating to release, such as calcium requirements and kinetics, they differed markedly in their response to different stimuli. In this and all other aspects of heterogeneity examined, the neonatal and adult skin mast cells behave identically.

The classical secretagogue for studying histamine release in murine systems has been the polyamine, compound 48/80. Many thousands of studies have been published with this material which, until now, has been completely inert in human mast cells or basophils (40). Thus, the sensitivity of skin mast cells to this secretagogue is of considerable interest to the field, although its biologic significance is unclear. The three other secretagogues that act on skin mast cells, but not on other mast cells, have more biologic interest. As previously reported, morphine caused histamine release only from skin mast cells and this, perhaps, helps us to understand the potential role of the skin in systemic reactions. The *i.v.* use of morphine in man, especially during the general anesthesia used in cardiac surgery, almost uniformly leads to

increased blood histamine levels. Yet of all mast cells studied to date, including those of the heart, only the skin mast cell responds to this stimulus, suggesting that localized cutaneous mediator release may play a role in systemic reactions (2, 27, 41, 42).

Recent work has focused on the relationship between the nervous system, acting via neuropeptides, and various inflammatory responses. Substance P has been implicated in these studies because it is a potent secretagogue for murine mast cells (43, 44). Although others have claimed that substance P can cause release from basophils, we have not been able to confirm this observation, nor has it proved to be active against mast cells obtained from the lung or the gut (45). However, substance P clearly acts on the skin mast cells and allows us to speculate about a neuroinflammatory link, at least for the human skin.

Although human basophils failed to respond to morphine or substance P, they did respond to C5a and FMLP, small peptides that are biologically relevant molecules (46–48). Interestingly, the basophil response to C5a is characterized by a relative predominance of histamine release with little LTC₄ secretion whereas the smaller bacterial chemotactic factor causes release of both histamine and LTC₄. Both of these stimuli are completely inactive on mast cells obtained from lung or gut but, as shown in this report, are active on human skin mast cells. Thus, the skin mast cell resembles the basophil more than the relatively inert mast cells lining the airways and gut. Whatever the reason for these differences among mast cells, they are clearly of significance in understanding the pathogenetic potential of these cells and will almost surely be reflected in differences in the pharmacologic control of these cells. It is already known that each of these releasing stimuli, in human basophils, is controlled differentially with respect to agonists (49).

One of the most convincing markers of heterogeneity in the murine system is that the connective tissue mast cell fails to make LTC₄, generating rather the cyclo-oxygenase product, PGD₂, whereas the mucosal mast cell makes predominantly LTC₄ (50, 51). This limited repertoire of arachidonic acid products has been maintained in murine, bone marrow-derived, nonadherent tissue culture cell lines that are supposed to represent the different types of mast cells (52). Our previous studies with the lung and gut mast cells demonstrated that these cells made substantial quantities of both PGD₂ and LTC₄ (6, 15), whereas the human basophil generates LTC₄ with little or no PGD₂ production (34). On this basis, it has been suggested that the basophil was a counterpart of the murine mucosal mast cell. The skin mast cell represents a third pattern: it generates primarily PGD₂, producing less than 5% of the LTC₄ produced by lung or intestinal mast cells. Thus it would appear that the skin mast cell is a counterpart of the murine connective tissue mast cell, at least with regard to arachidonic acid metabolism. The finding that PGD₂ is the predominant metabolite of arachidonic acid in the skin mast cell is consistent with observations in patients with systemic mastocytosis who have elevated serum levels of histamine and PGD₂, but not of LTC₄ (53). In addition, we have shown that histamine and PGD₂ are the predominant mediators found in fluid obtained from skin blisters after antigen challenge (54). Thus, human skin mast cells differ from

other human mast cells in their preferential use of the cyclo-oxygenase pathway of arachidonic acid metabolism, with little metabolism occurring via the lipoxygenase pathway.

In summary, we have described a very convenient technique to prepare human mast cells from adult skin, which may be studied in suspension, either impure or simply purified to homogeneity. These mast cells are different from those described previously in a variety of parameters that promise to be of important pathogenetic and therapeutic significance.

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REFERENCES

- Bienenstock, J., A. D. Befus, F. Pearce, J. Denburg, and R. Goodacre. 1982. Mast cell heterogeneity: derivation and function, with emphasis on the intestine. *J. Allergy Clin. Immunol.* 70:407.
- Enerbäck, L. 1966. Mast cells in rat gastrointestinal mucosa. I. Effects of fixation. *Acta Pathol. Microbiol. Scand.* 66:289.
- Enerbäck, L. 1966. Mast cells in rat gastrointestinal mucosa. II. Dye binding and metachromatic properties. *Acta Pathol. Microbiol. Scand.* 66:303.
- Pearce, F. L. 1982. Functional heterogeneity of mast cells from different species and tissues. *Klin. Wochenschr.* 60:954.
- Lee, T. D., M. Sweiter, J. Bienenstock, and A. D. Befus. 1985. Heterogeneity in mast cell populations. *Clin. Immunol. Rev.* 4:143.
- Fox, C. C., A. Kagey-Sobotka, R. P. Schleimer, S. P. Peters, D. W. MacGlashan, and L. M. Lichtenstein. 1985. Mediator release from human basophils and mast cells from lung and intestinal mucosa. *Int. Arch. Allergy Appl. Immunol.* 77:130.
- Fox, C. C., A. M. Dvorak, S. P. Peters, A. Kagey-Sobotka, and L. M. Lichtenstein. 1985. Isolation and characterization of human intestinal mucosal mast cells. *J. Immunol.* 135:483.
- Schulman, E. S., A. Kagey-Sobotka, D. W. MacGlashan, N. F. Adkinson, S. P. Peters, R. P. Schleimer, and L. M. Lichtenstein. 1983. Heterogeneity of human mast cells. *J. Immunol.* 131:1936.
- Irani, A. A., N. M. Schechter, S. S. Craig, G. DeBlois, and L. B. Schwartz. 1986. Two types of mast cells that have distinct neutral protease compositions. *Proc. Natl. Acad. Sci. USA* 83:4464.
- Benyon, R. C., M. K. Church, L. S. Clegg, and S. T. Holgate. 1986. Dispersion and characterization of mast cells from human skin. *Int. Arch. Allergy Appl. Immunol.* 79:332.
- Casale, T. B., S. Bowman, and M. Kaliner. 1984. Induction of human cutaneous mast cell degranulation by opiates and endogenous opioid peptides: evidence for opiate and nonopiate receptor participation. *J. Allergy Clin. Immunol.* 73:775.
- Clegg, L. S., M. K. Church, and S. T. Holgate. 1985. Histamine secretion from human skin slices induced by anti-IgE and artificial secretagogues and effects of sodium chromoglycate and salbutamol. *Clin. Allergy* 15:321.
- Ebertz, J. M., J. M. Hermens, J. C. McMillan, H. Uno, C. Hirshman, and J. M. Hanifin. 1986. Functional differences between cutaneous mast cells and basophils: a comparison of morphine-induced histamine release. *Agents Actions* 18:455.
- Tharp, M. D., R. T. Suvunrunsi, and T. J. Sullivan. 1983. IgE-mediated release of histamine from human cutaneous mast cells. *J. Immunol.* 130:1896.
- MacGlashan, D. W., R. P. Schleimer, S. P. Peters, E. S. Schulman, G. K. Adams, H. H. Newball, and L. M. Lichtenstein. 1982. Generation of leukotrienes by purified human lung mast cells. *J. Clin. Invest.* 70:747.
- Goldstein, B., M. Dembo, A. K. Sobotka, and L. M. Lichtenstein. 1979. Some invariant properties of IgE-mediated basophil activation and desensitization. *J. Immunol.* 123:1873.
- Peters, S. P., E. S. Schulman, R. P. Schleimer, D. W. MacGlashan, H. H. Newball, and L. M. Lichtenstein. 1982. Dispersed human lung mast cells. *Am. Rev. Respir. Dis.* 126:1034.
- Schulman, E. S., D. W. MacGlashan, S. P. Peters, R. P. Schleimer, H. H. Newball, and L. M. Lichtenstein. 1982. Human lung mast cells: purification and characterization. *J. Immunol.* 129:2662.
- Lichtenstein, L. M. and A. G. Osler. 1964. Studies on the mechanisms of hypersensitivity phenomena. IX. Histamine release from human leukocytes by ragweed pollen antigen. *J. Exp. Med.* 120:507.
- Siraganian, R. P. 1974. An automated continuous-flow system for the extraction and fluorometric analysis of histamine. *Analyt. Biochem.* 57:383.
- Hayes, E. C., D. L. Lomardo, Y. Girard, A. L. Maycock, J. Rokach, A. S. Rosenthal, R. N. Young, R. W. Eyan, and H. J. Zweernick. 1983. Measuring leukotrienes of slow reacting substance of anaphylaxis: development of a specific radioimmunoassay. *J. Immunol.* 131:429.
- Schulman, E. S., H. H. Newball, F. A. Demers, F. A. Fitzpatrick, and N. F. Adkinson. 1981. Anaphylactic release of thromboxane A₂, prostaglandin D₂ and prostacyclin from human lung parenchyma. *Am. Rev. Respir. Dis.* 124:402.
- Hubbard, W. C., C. L. Litterest, M. C. Liu, E. R. Bleecker, J. C. Eggleston, T. L. McLeMore, and M. R. Boyd. Profiling of prostaglandin biosynthesis in biopsy fragments of human lung carcinoma and human lung tissue by capillary gas chromatography-negative ion chemical ionization mass spectrometry. *Prostaglandins*. In press.
- Rouzer, C. A., A. L. Scott, A. L. Hamill, and Z. A. Cohn. 1982. Synthesis of leukotriene C and other arachidonic acid metabolites by mouse pulmonary macrophages. *J. Exp. Med.* 155:720.
- Lewis, R. A., K. F. Austen, J. M. Drazen, D. A. Clark, A. Marfat, and E. J. Corey. 1980. Slow reacting substances of anaphylaxis: identification of leukotriene C-1 and D from human and rat sources. *Proc. Natl. Acad. Sci. USA* 77:3710.
- Marone, G., A. Kagey-Sobotka, and L. M. Lichtenstein. 1981. IgE-mediated histamine release from human basophils: differences between antigen-E and anti-IgE-induced secretion. *Int. Arch. Allergy Appl. Immunol.* 65:339.
- Hermans, J. M., J. M. Ebertz, J. M. Hanifin, and C. A. Hirshman. 1985. Comparison of histamine release in human skin mast cells induced by morphine, fentanyl and oxymorphone. *Anesthesiology* 62:124.
- Devillier, P., D. Regoli, A. Asseraf, B. Descours, J. Marsac, and M. Renoux. 1986. Histamine release and local responses of rat and human skin to substance-P and other mammalian tachykinins. *Pharmacology* 32:340.
- Gillespie, E., and L. M. Lichtenstein. 1975. Histamine release from human leukocytes: relationships between cyclic nucleotide, calcium and antigen concentrations. *J. Immunol.* 115:1572.
- Foreman, J. C., C. C. Jordan, and W. Piotrowski. 1982. Interaction of neurotensin with substance P receptor mediating histamine release from rat muscle cells and the flare in human skin. *Br. J. Pharmacol.* 77:531.
- Fjeller, B., and O. Hagermark. 1981. Studies on pruritogenic and histamine-releasing effects of some putative peptide neurotransmitters. *Acta Derm. Venereol.* 61:245.
- Jorizzo, J. L., A. A. Coutts, R. A. Eachy, and M. W. Greaves. 1983. Vascular responses of human skin to injection of substance P and mechanism of action. *Eur. J. Pharmacol.* 87:67.
- Piotrowski, W., M. A. Devoy, C. C. Jordan, and J. C. Forman. 1984. The substance P receptor on rat mast cells and in human skin. *Agents Actions* 14:420.
- Schulman, E. S., D. W. MacGlashan, R. P. Schleimer, S. P. Peters, A. Kagey-Sobotka, H. H. Newball, and L. M. Lichtenstein. 1983. Purified human basophils and mast cells: current concepts of mediator release. *Eur. J. Respir. Dis.* 64:53.
- Lawrence, I. D., J. A. Warner, R. Bascom, A. Kagey-Sobotka, and L. M. Lichtenstein. 1987. Heterogeneity of human mast cells. *J. Allergy Clin. Immunol.* 79:179.
- Nakano, T., T. Sonoda, C. Hayashi, A. Yamatodani, Y. Kanayama, T. Yamamura, H. Asai, T. Yonezawa, Y. Kitamura, and S. J. Galli. 1985. Fate of bone marrow-derived cultured mast cells after intracutaneous, intraperitoneal and intravenous transfer into genetically mast cell deficient w/w^o mice. *J. Exp. Med.* 162:1025.
- Lichtenstein, L. M., J. C. Foreman, M. C. Conroy, G. Marone, and H. H. Newball. 1979. Differences between histamine release from rat mast cells and human basophils and mast cells. In *The Mast Cell, Its Role in Health and Disease*. J. Pepys and A. M. Edwards, eds. Pitman Medical Publishing Co., Ltd., Kent, England, p. 83.
- Peters, S. P., E. S. Schulman, D. W. MacGlashan, R. P. Schleimer, H. H. Newball, and L. M. Lichtenstein. 1982. Dispersed human lung mast cells: pharmacologic aspects and comparison with human lung tissue fragments. *Am. Rev. Respir. Dis.* 126:1034.
- Marone, G., R. Gugliano, G. Lembo, and F. Ayala. 1986. Human basophil releasability. I. Age related changes in basophil releasability. *J. Allergy Clin. Immunol.* 77:377.
- Church, M. K., G. J.-K. Pao, and S. T. Holgate. 1982. Characterization of histamine secretion from mechanically dispersed human lung mast cells: effects of anti-IgE, calcium ionophore A23187, compound 48/80 and basic polypeptides. *J. Immunol.* 129:2116.
- Tharp, M. D., A. Kagey-Sobotka, C. C. Fox, G. Marone, L. M. Lichtenstein, and T. J. Sullivan. 1987. Functional heterogeneity of human mast cells from different anatomic sites: *In vitro* responses to morphine sulfate. *J. Allergy Clin. Immunol.* 79:646.
- Hirshman, C. A., R. A. Edelstein, J. M. Ebertz, and J. M. Hanifin. 1985. Thiobarbiturate-induced histamine release in human skin mast cells. *Anesthesiology* 83:353.

43. Johnson, A. R., and E. G. Erdos. 1973. Release of histamine from mast cells by vasoactive peptides. *Proc. Soc. Exp. Biol. Med.* 142:1252.
44. Fjellner, B., and O. Hagermak. 1981. Studies on prurigenic and histamine-releasing effects of some putative peptide neurotransmitters. *Acta Derm. Venerol.* 61:245.
45. Ali, H., K. B. P. Leung, F. L. Pearce, N. A. Hayes, and J. C. Foreman. 1986. Comparison of the histamine-releasing action of substance P on mast cells and basophils from different species and tissues. *Int. Arch. Allergy Appl. Immunol.* 79:418.
46. Grant, J. A., E. Dupree, and A. S. Goldman. 1975. Complement mediated release of histamine release from human leukocytes. *J. Immunol.* 114:1101.
47. Hook, W. A., R. P. Siriganian, and S. M. Nahl. 1975. Complement mediated release from human basophils. Generation of activity in human serum. *J. Immunol.* 114:1185.
48. Siriganian, R. P., and W. A. Hook. 1977. Mechanism of histamine release by formyl-methionine-containing peptides. *J. Immunol.* 119:2078.
49. Newball, H. H., and L. M. Lichtenstein. 1983. Pharmacologic modulation of the immune release of mediators from mast cells and basophils. In *Lung Biology in Health and Disease: Immunopharmacology of the Lung*. Vol. 19. H. H. Newball, ed. Marcel Dekker, New York, pp 1-23.
50. Lewis, R. A., K. F. Austen, J. M. Drazen, D. A. Clark, A. Marfat, and E. J. Corey. 1980. Slow reacting substances of anaphylaxis: identification of leukotrienes C-1 and D from human and rat sources. *Proc. Natl. Acad. Sci. USA* 77:3710.
51. Lewis, R. A., N. A. Soter, P. T. Diamond, K. F. Austen, J. A. Oates, and L. J. Roberts. 1982. Prostaglandin D₂ generation after activation of rat and human mast cells with anti-IgE. *J. Immunol.* 129:1627.
52. Nagao, K., K. Tokoro, and S. A. Aaranson. 1981. Continuous lines of basophil/mast cells derived from normal mouse bone marrow. *Science* 212:333.
53. Roberts, L. J., B. J. Sweetman, R. A. Lewis, K. F. Austen, and J. A. Oates. 1980. Increased production of prostaglandin D₂ in patients with systemic mastocytosis. *N. Engl. J. Med.* 303:1400.
54. Reshef, A., A. Kagey-Sobotka, L. M. Lichtenstein, and P. S. Norman. 1987. The pattern and kinetics of mediators in acute and late phase response (LPR) in human skin. *J. Allergy Clin. Immunol.* 79:248.