Isolation and Purification of Human Cutaneous Mast Cells

Mustafa Şenol, MD[1], İ. Halil Özerol, MD[2], Asha V. Patel, MS[1], David P. Skoner, MD[1]

Mast cells play an important role in immediate and late type allergic reactions. Most of our knowledge on properties of mast cells was derived from animal models and these methods have some limitations. We aimed to describe a modified and improved in vitro method for isolation and purification of human skin mast cells. Human neonatal foreskins were digested and isolated by using an enzymatic dispersion procedure. Then they were purified by density centrifugation through Percoll gradients. Spontaneous and ionophore-induced histamine releases from both unpurified and purified mast cell preparations were assayed by enzyme-linked immunosorbent assay. This technique produced reproducible and satisfactory results for isolation, purification, and stimulation of human skin mast cells in a good yield with an excellent viability. In this article, this detailed in vitro method for studies on human skin mast cell was described. It is hoped that newly-developed in vitro and in vivo methods will increase the knowledge about the role of human skin mast cells. [Journal of Turgut Özal Medical Center 1996;3(3):143-150]

Key Words: *Human skin mast cell, digestion, purification, ionophore*

Kutanöz mast hücrelerinin elde edilmesi ve saflaştırılması

Mast hücreleri erken ve geç tip allerjik reaksiyonlarda önemli rol oynamaktadır. Mast hücreleri hakkındaki bilgilerimizin çoğu, in vitro hayvan modelleriyle yapılan çalışmalara dayanmaktadır, fakat bu metodların birtakım mahzurları ve kısıtlılıkları bulunmaktadır. Bu çalışmada; insan deri mast hücrelerinin elde edilmesi ve saflaştırılması konusunda kullanışlı bir metod geliştirilmesi amaçlanmıştır. Yenidoğan sünnet derilerinin enzimatik bir işlemle parçalanmasıyla elde edilen mast hücreleri, Percoll yoğunluk farkına dayalı santrifügasyon yöntemiyle saflaştırıldı. Ham ve saflaştırılmış mast hücrelerinden, spontan ve kalsiyum iyonoforla uyarılma sonucu salgılanan histamin miktarları ELISA yöntemiyle ölçüldü. Bu metod; insan deri mast hücrelerinin elde edilmesi, saflaştırılması ve stimülasyonu konusunda başarılı sonuçlar vermiştir. Elde edilen mast hücreleri sayı ve canlılık açısından yeterlidir. Bu makalede, bahsedilen metod ayrıntılı şekilde anlatılmıştır. Yeni geliştirilecek in vivo ve in vitro metodlarla mast hücreleri hakkındaki bilgilerimizin daha da artacağı umulmaktadır. [Turgut Özal Tıp Merkezi Dergisi 1996;3(3):143-150]

Anahtar Kelimeler: *Kutanöz mast hücresi, enzimatik parçalama, saflaştırma, iyonofor*

Mast cells are tissue effectors of immunologic and nonimmunologic immediate hypersensitivity reactions (1-3). Besides this primary function, they may be involved in the neuronal control of local blood flow, angiogenesis, and fibroblast proliferation, and can carry information to the organism about external noxious agents (4,5). Mast cells from different species and different anatomical sites are highly heterogeneous. This heterogeneity has been studied widely over the past decades, especially on the animal models (6-7).

[1] University of Pittsburgh School of Medicine Department of Pediatrics Section of Allergy/Immunology, Pittsburgh, PA 15213, USA

[2] University of Nebraska Medical Center Department of Pathology/Microbiology Omaha, NE 68198, USA

In rats and mice, mast cells have been classified according to their anatomical position; MMC for mucosal mast cells, CTMC for connective tissue mast cells (8). In humans, they have been named according to their neutral protease contents; MC_T for those contain only tryptase and MC_{TC} for those contain tryptase, chymase, carboxypeptidase, and cathepsin-G (4,9-11). MC_{TC} cells are predominant in the skin and the gastrointestinal submucosa, whereas MC_T cells are the predominant type found in the lung and the intestinal mucosa (7,9,12).

Human cutaneous mast cells are located mainly in the papillary dermis around capillaries, dermal appendages, nerves, and lymphatics (4,13). In human skin, there are about 7,000 to 10,000 mast cell/mm (3), but the number may vary with anatomical site, age, or existence of disease (4,10). Microscopically, cutaneous mast cells appear as round, oval, or even dendritic cells. Within the cytoplasm, there are 50-200 dense granules that often mask the nucleus. These secretory granules contain the pre-formed mast cell mediators and are stained by cationic dyes metachromatically (10,14, 15). Human mast cells contain 1.5 to 10 pg histamine per cell (1,16).

Although useful, animal models have some limitations, because rodent mast cells appear different in their morphology, mediator content, staining properties, dependence on T lymphocyte-derived factors or microenvironment for differentiation, sensitivity to different chemical agents, and response to immunologic and non-immunologic stimuli. Same kinds of limitations can be considered about in vitro human leukocyte (basophil) and lung systems. The circulating basophil is distinctly different from mast cell. Pulmonary and gastrointestinal mast cells also may not be entire representative of all the mast cells (17). These disadvantages made the use of dispersed human skin mast cells essential and some new techniques were developed for proteolytic dispersion of viable cutaneous mast cells and their density gradient purification (17-22).

In this study, we described a detailed, and a partly modified in vitro model for studies on human skin mast cell function. This technique permits the reproducible isolation and purification of human cutaneous mast cells in a good yield with an excellent viability.

MATERİALS AND METHODS

Materials: The following reagents and enzymes were used in the experiments: bovine serum albumine (BSA), $CaCl_2$, calcium ionophore A23187, crystal violet, deoxyribonuclease (DNAse) (bovine pancreas), dimethyl sulfoxide (DMSO), heparin, hyaluronidase, KCl, N-2-hydroxyethylpiperazine-N`-2-ethanesulfonic acid (HEPES), ouabain, perchloric acid, Percoll, piperazine-N,N-bis-2-ethanesulfonic acid (PIPES), (Sigma, St. Louis, MO); collagenase A, Dulbecco`s modified Eagle`s medium (DMEM), fetal calf serum (FCS), fungizone, gentamycin, L-glutamine, penicillin/streptomycin (pen/strep), (Gibco, Grand Island, NY); dextrose, NaCl, $MgCl_2$, Na_3PO_4, toluidine blue, trypan blue (Fisher Scientific, Pittsburgh, PA); nylon mesh (150 μm-pore size) (Tetko, Manor, NY); histamine enzyme-linked immunosorbent assay (ELISA) kit (Immunotech, Westbrook, MA).

***Buffers and solutions*:** The following buffers and solutions were prepared:

1- Foreskin medium. DMEM supplemented with HEPES 8.4 mM, L-glutamine 1%, pen/strep 80 U/ml, fungizone 2 μg/ml, gentamycin 20 μg/ml, pH 7.2. (To make 500 ml: 500 ml DMEM, 1 g or 10 ml 100X HEPES, 5 ml L-glutamine, 1 ml pen/strep, 1 ml fungizone, 0.5 ml gentamycin. Mix above, adjust pH to 7.2, filter aseptically with 22 μm filter, add 10 ml to 50-ml polypropilene tubes, store at 4°C, make fresh monthly).

2- Sodium phosphate buffer. Monobasic Na_3PO_4 (mw 138.0) 50 mM and dibasic Na_3PO_4 (mw 142.0) 50 mM, in distillated water. (To make 500 ml: 500 ml ddH_2O, 3.45 g monobasic Na_3PO_4, 3.55 g dibasic Na_3PO_4, mix above, store at 4°C, make fresh monthly).

3- Blank buffer. Na_3PO_4 2.5 mM, NaCl 135 mM, KCl 3.76 mM, HEPES 2.1 mM, glucose 5.5 mM, in distillated water, pH 7.2. (To make 500 ml: 475 ml ddH_2O, 25 ml sodium phosphate buffer, 3.95 g NaCl, 0.14 g KCl, 0.24 g HEPES, 0.5 g glucose. Mix above, adjust pH to 7.2, store at 4°C, make fresh weekly).

4- Wash buffer. Blank buffer supplemented with heparin 7 μg/ml, DNAse 0.1 mg/ml, FCS 5%. (To make 100 ml: 100 ml blank buffer, 0.7 mg heparin,

10 mg DNAse, 5 ml FCS. Mix above, store at 4°C, make fresh daily).

5- Digestion buffer. Blank buffer supplemented with DNAse 1 mg/ml, hyaluronidase 1 mg/ml, collagenase 0.75 mg/ml, heparin 10 μg/ml, HEPES 10 mM, $CaCl_2$ 1 mM, $MgCl_2$ 1 mM, BSA 1%, pH 7.2. (To make 100 ml: 100 ml blank buffer, 1 mg heparin, 0.24 g HEPES, 0.015 g $CaCl_2$, 0.020 g $MgCl_2$. Mix above, adjust pH to 7.2, add 1 g BSA, stir, store at 4°C, make fresh daily. Just prior to use, add 20 mg DNAse, 20 mg hyaluronidase, and 15 mg collagenase to 20 ml of digestion buffer).

6- Histamine release buffer stock solutions.

a-5X PIPES (125 mM). Add 18.9 g PIPES to 500 ml of ddH_2O, adjust pH to 7.2, store at 4°C, make fresh monthly.

b-10X NaCl (1 M). Add 29.2 NaCl to 500 ml of ddH_2O, store at 4°C, make fresh monthly.

c-100X KCl (0.5 M). Add 3.7 KCl to 100 ml of ddH_2O, store at 4°C, make fresh monthly.

d-100X $MgCl_2$ (40 mM). Add 0.8 g $MgCl_2$ to 100 ml of ddH_2O, store at 4°C, make fresh monthly.

7- Histamine release buffer. Distillated water containing PIPES 25 mM, NaCl 100 mM, KCl 5 mM, $MgCl_2$ 0.4 mM, glucose 5.5 mM, BSA 0.1%. (To make 100 ml: 20 ml 5X PIPES, 10 ml 10X NaCl, 1 ml 100X KCl, 1 ml 100X $MgCl_2$, 2 ml 5% BSA, 0.1 g glucose, add ddH_2O to bring volume to 100 ml, store at 4°C, make fresh daily).

8- 10X $CaCl_2$ (10 mM stock solution). Add 0.147 g $CaCl_2$ to 100 ml of ddH_2O, store at 4°C, make fresh monthly).

9- Calcium ionophore A23187 (1 mM stock solution). Dissolve 1 mg calcium ionophore in 1 ml of DMSO, divide to 50 μl aliquots, store at -20°C. Just prior to use, add 30 μl stock solution to 970 ml of histamine release buffer *(calcium ionophore solution).*

10- 10X mast cell medium (10X MCM). HEPES 16 mM, NaCl 92.6 mM, KCl 2.3 mM, glucose 3.5 mM, in distillated water, pH 7.2. (To make 100 ml: 100 ml ddH_2O, 0.6 g HEPES, 8.6 g NaCl, 0.28 g KCl, 1 g glucose, adjust pH to 7.2, store at 4°C, make fresh monthly).

11- Toluidine blue (0.1%). Add 25 mg toluidine blue to 25 ml of 30% ethanol, adjust pH to 1, store at room temperature, make fresh monthly.

12- Crystal violet (0.2%). Add 50 mg crystal violet and 1.25 ml acetic acid to 23.75 ml of ddH_2O, stir and filter, store at room temperature, make fresh monthly.

13- Trypan blue (0,3%). Dissolve 1 g trypan blue in 100 ml of ddH_2O, filter or centrifuge and store at 4°C, make fresh monthly *(1% stock solution).* Add 3 ml stock solution to 7 ml of balanced salt solution or histamine release buffer, make fresh daily *(daily working solution).*

Mast cell isolation: Foreskins obtained at elective circumcision from infants aged 3 to 7 days were placed in 10 ml of foreskin medium, stored at 4°C, and used within 48 hr of excision. After disgarding the foreskin medium, the tissue was dried on steril gauze, weighed, and rinsed with 20 ml of 70% ethanol. The tissue was choppped finely (1 to 2 mm) with flame-sterilized forceps and scissors in a Petri dish containing 5 ml of wash buffer and washed twice in 10 ml of wash buffer by centrifuging at 1700 rpm for 5 min at 12°C. The resulting fragments (<2.0 g) were then incubated in 20 ml of digestion buffer for 1 hr at 37°C in a shaking water bath by agitating every 15 min. The digested tissue was filtered over sterile nitex cloth into a 50-ml tube and the resultant cell suspension was washed twice in 20 ml of histamine release buffer in the same manner mentioned above. Undigested tissue was subjected to a second identical digestion and cell separation. Cells obtained from each digestion were pooled and washed once in histamine release buffer. After resuspending in 2 ml of histamine release buffer, equal amounts (15 μl) of cell suspension and dye were mixed. Mast cell number and purity were determined by ligt microscopy on a Neubauer hemacytometer after staining with toluidine blue or crystal violet (especially to determine the cells other than mast cells). Mast cells in all squares were counted and the total mast cell number was calculated as follows:

Total mast cell number = the number of counted mast cells x 2 (dilution coefficient) x 1.11 (correction coefficient) x 1000 x 2 (ml of suspension).

Cell count was adjusted to 12,500 mast cells/ml (1000 mast cells/80 µl) by adding appropriate amount of histamine release buffer and cells were placed on ice or stored at 4°C until the experiments.

Mast cell purification: Percoll was prepared by mixing 4.25 parts of Percoll and 1 part of 10X MCM and further diluted with histamine release buffer to give final concentrations of 90%, 80%, and 50% Percoll. Gradients were prepared in 50-ml polypropilene tubes by sequental and slow overlayering 10 ml of each of 90%, 80%, and 50% Percoll. The dispersed cells (4 to 5 x 10^5 mast cells in 10 ml of histamine release buffer) were layered slowly over the 50% layer of Percoll and centrifuged at 1000 rpm for 30 min at 12°C. The supernatant, interfaces, and pellet were collected by aspiration with a 1-ml air-displacement pipette and washed twice in histamine release buffer. The number and purity were determined as described above and cell number was adjusted to 12,500 mast cell/ml.

***Histamine release assay*:** 1000 mast cells (unpurified or purified) were used in each experimental tube. All experiments were performed in triplicate. Samples of 80 µl containing 1000 mast cells were placed in 1.5-ml Eppendorf tubes and 10 µl of 10X$CaCl_2$ (1 mM final concentration) was added to each tube. Then 10 µl of histamine release buffer (to spontaneous histamine release tubes) or 10 µl of calcium ionophore solution (1 µM final concentration) (to stimulated histamine release tubes) were added. 0 hr control tubes were set aside on ice and 900 µl of ice-cold histamine release buffer was added to each tube. After incubation at 37°C for 30 min, reactions were terminated by the addition of 900 µl of ice-cold histamine release buffer. Then tubes were centrifuged at 2500 rpm for 2 min. Supernatants (1:10 diluted) were removed and stored at -70°C until histamine assay. Total histamine was determined from samples (1000 mast cells in 1 ml of histamine release buffer) which were boiled 10 min, frozen and thawed for 5 times, or treated with perchloric acid (3%) (1000 mast cells in 960 µl of histamine release buffer and 40 µl of 70% perchloric acid) to obtain all the histamine content.

***Histamine analyses*:** Supernatants were assayed for histamine by using a commercial enzyme-linked immunosorbent assay kit (23,24). 100 µl of standards (duplicate) or spontaneous histamine release supernatants (triplicate, 1:10 diluted) and 25 µl of induced and total histamine release supernatants and 75 µl histamine release buffer (triplicate, 1:40 diluted) were added to acylation tubes provided. After adding 50 µl of acylation buffer, tubes were incubated at room temperature for 30 min. Then 50 µl of acylated standards or samples and 200 µl of histamine-acetylcholinesterase conjugate were added to each well of 96-well plate provided. After incubating at 4°C for 18 hr, wells were rinsed three times with 1:20 diluted wash solution and 200 µl of chromogenic substrate added to each well. The plate was incubated at room temperature in a dark place for 1 hr and 50 µl of stop solution added to each well to stop the enzymatic reaction. Then the absorbances of wells were read at 405 nm by using a microtiter plate reader. Data was processed and end results were calculated on computer by using Kineticalc and Lotus programs. The histamine in the supernatants (ng/ml) was divided the total histamine content of the mast cells to determine the percentage of histamine released during the experiments. Spontaneous histamine release was substracted from stimulated histamine release to give the net stimulated histamine release. Results were expressed as percentage of total histamine content.

Cell viability: Cell viability was assessed by trypan blue exclusion in parallel with all experiments (25).

Statistical analysis: All results were expressed as mean±SEM. Significance of differences was evaluated by using two-tailed Student's t-test for paired data. Differences were considered significant when probability (p) was less than 0.05.

RESULTS

***Cell isolation*:** Dispersion of human neonatal foreskins with an enzymatic digestion (collagenase, DNAse, and hyaluronidase) yielded an average of 3.45±0.55 x 10^5 mast cells/g wet weight of skin (n=25). The initial purity of mast cells was 3.1±0.8 %, and the major contaminating nucleated cells were fibroblasts and capillary endothelial cells with some lymphocytes.

Cell purification: Human neonatal foreskin mast cells were enriched by density centrifugation through Percoll gradients (Table 1). The highest

mast cell purity, 68.7±6.2 %, was obtained at the bottom of the tubes and these cells were used in the stimulation experiments. Approximately 20% of the total mast cells were recovered in the pellet and they were contaminated with erythrocytes and some fibroblasts and endothelial cells. 51.2±4.9% and 34.6±3.8% purity were obtained from the 90/80% and 80/50% Percoll interfaces, respectively. Contaminating cells were fibroblasts, endothelial cells, and some lymphocytes. The purity was less than 1% at the top of the tubes.

Histamine content of mast cells: Unpurified mast cells treated by freezing and thawing 5 times yielded 1.5±0.7 pg/cell, cells boiled for 10 min yielded 6.3±1.4 pg/cell, and cells treated with 3% perchloric acid yielded 6.7±1.5 pg/cell histamine (n=5). We preferred to use boiling method in subsequental experiments to obtain the total histamine content. The average histamine content of unpurified mast cells was 6.5±2.3 pg/cell (n=25). This content was 6.2±1.9 pg/cell for 50/80% interface, 6.4±1.8 pg/cell for 80/90 % interface, and 6.1±1.5 pg/cell for the pellet (n=7) (Table 1).

Histamine release from mast cells: Spontaneous, calcium ionophore A23187-induced, and net histamine releases from unpurified and purified mast cells are shown in Figure 1. Spontaneous histamine releases from unpurified and purified mast cells were 12.02±2.19% and 3.77±0.64% at the beginning, whereas they were 29.18±4.67% and 5.52±1.43% at the end of incubation period, respectively. Calcium ionophore-induced releases from unpurified and purified cells were 38.74±6.11 % and 14.06±2.63 % at the beginning and 68.97±8.75 % and 41.67±7.04 % at the end of incubation, respectively. As seen in the figure, all the histamine releases from unpurified mast cells were higher than those of purified ones and calcium ionophore caused rapid and significant ($p<0.001$) histamine releases from both kinds of cells at the beginning and at the end of incubation period.

Cell viability: Viability of mast cells assessed by staining with trypan blue in both from unpurified and purified mast cell preparations was higher than 95% in all experiments.

DİSCUSSİON

Mast cells play an important role in the immunologic and non-immunologic inflammatory reactions. Mast cells are higly heterogeneous and the reason for this heterogeneity is still unclear (2,3). Mast cells derived from different mammalian species vary in their morphological characteristics and secretory responses to different stimuli. Morever, mast cells from different anatomical sites within the same organism express different morphologic, biochemical, and functional properties (19,20). Whatever the reason of this heterogeneity, it is clearly significant in understanding pathobiology of these cells and will be reflected in differences in the pharmacologic control of mast cells (8,19).

Mast cells have been a popular and interesting model system for studying stimulus-secretion coupling and secretory responses to various stimuli (28). Most of the detailed knowledge on mast cell function was derived from in vitro studies performed by using isolated rodent mast cells, human basophils, and human pulmonary and gastrointestinal mast cells (17). Human skin mast cells differ from other kinds of human mast cells in being responsive to immunologic and a wide variety of non-immunologic stimuli including calcium ionophore A23187, compound 48/80, poly-L-lysine, morphine, substance P, somatostatin, vasoactive intestinal peptide (VIP), anaphylatoxin C5a, f-met peptide. Except calcium ionophore, all of these non-immunologic agents have no effect on pulmonary and gastrointestinal mast cells (5,19). Immunologic and non-immunologic stimuli activate skin mast cells by different mechanisms (5). The function of skin mast cells has been studied for the

Table 1. Purification of human neonatal foreskin mast cells by Percoll density centrifugation and their histamine content.

	Percentage of total nucleated cells				Histamine content	
Location	Mast cells	Fibroblasts	Endothelial cells	Others	(pg/cell)	n
Crude cells	3.1±0.8	34.5±3.4	53.3±4.7	9.1±1.8	6.5±2.3	25
Top of the tube	1<	43.7±3.5	48.4±4.1	8.2±1.3	-	7
50/80 interface	34.4±3.8	31.2±3.9	28.1±4.2	6.3±1.7	6.2±1.9	7
80/90 interface	51.2±4.9	20.7±3.1	22.6±3.6	4.5±1.6	6.4±1.8	7
Pellet	68.7±6.2	15.3±3.6	12.8±2.8	3.2±1.4	6.1±1.5	7

last three decades. Recently new enzymatic dispersion and rapid purification techniques have been developed (17-22).

We were able to isolate an average of 3.45±0.55 x 10^5 mast cells from 1 g wet neonatal foreskin tissue. These numbers have been changing from 4.15 x 10^5 to 1.61±0.10 x 10^6 mast cells/g wet weight of tissue in the previous studies (18,20). It seems possible to increase the isolated mast cell numbers by performing additional digestion processes.

In the present study, the initial purity of dispersed mast cells was 3.1±0.8 % with a contaminating dermal cell population of fibroblasts, endothelial cells, and a few lymphocytes. Epidermal cells were removed during cell filtration. In the literature, these values were changing between 4.2 % and 13.2 % with same kinds of contaminating cells (18-21). This variability can be explained by the differences in the experimental conditions and digestion procedures.

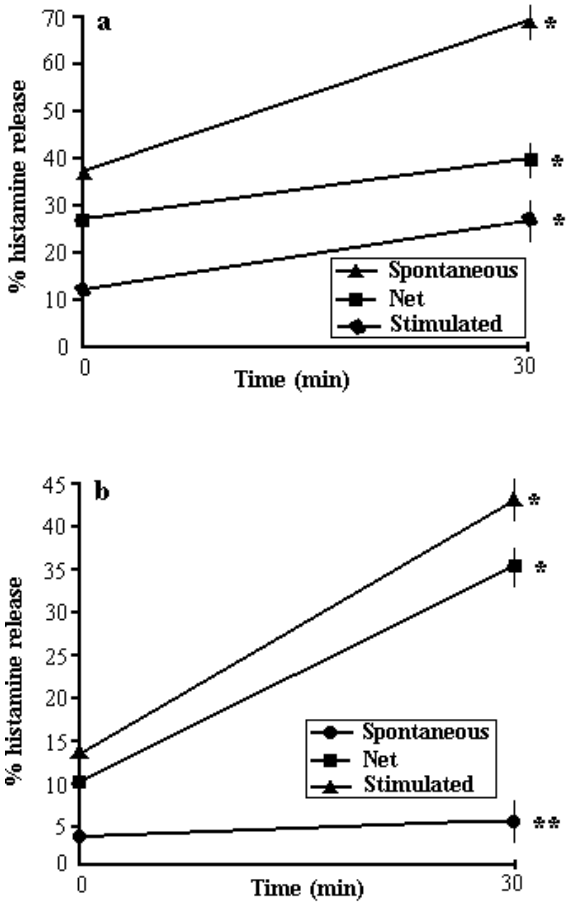

Figure 1. Spontaneous, calcium ionophore A23187-induced, and net histamine releases from (**a**) unpurified and (**b**) purified human cutaneous mast cells.

*, p<0.01 **, p<0.025

Some experimental studies on mast cells require pure preparations that are obtained by separation through density gradient centrifugation (28). The higher density of skin mast cells (>1.07 g/ml) compared with other cells allows the mast cell purification by a single-step density centrifugation (18). It can be possible by purification to characterize the responses of skin mast cells to many kinds of stimuli while minimizing the effect of other cells (19). Sucrose, Ficoll, Percoll, BSA, and Nycodenz were used for this purpose. Nycodenz is a relatively new material and is seldomly used for mast cells. Actually, Percoll is being utilized more often. The effect of purification media on the biological response of mast cells should be considered. High osmolarity media induces more important changes on the cell response than low osmolarity media. Percoll is a low-osmolar media (28). For that reason, we preferred Percoll as purification media.

We observed the highest purity from the pellets. Some of the previous reports have indicated similar results as ours (18,22), whereas some of them have reported that they got the highest purity from the interfaces between higher density gradients (19,21). In these studies, the degree of the observed highest purity was changing from 25% to 100% (18,19,21,22). This wide range probably should be due to some technical details like the numbers of interfaces, the speed, lenght, and temperature of centrifugation, the volume and the number of unseparated mast cells. The ratio of the cells recovered from the highest purity fraction to the total mast cells was fairly low as parallel with some previous studies (18). In some other reports, they have been able to recover higher proportions (19,22). The same considerations mentioned above can be thought for these differences.

There are different methods to obtain the total histamine content from the mast cells including boiling (17,19,20), freezing and thawing repeatedly (18,21), treating with perchloric (19) or trichloroacetic acids (22). Freezing and thawing 5 times failed to give sufficient results in our experiments. Boiling for 10 min and treating with

3% perchloric acid gave similar results. We used boiling method because of its simplicity, adequacy, and safety.

Human skin mast cells contain 1.5 to 10 pg histamine per cell (1,4,10,16). We obtained similar amounts of histamine from both unpurified and purified mast cells. Mast cells obtained from interfaces with different purities gave also similar amounts of histamine, but the histamine content decreased slightly after purification process as observed in a previous study (26) (Table 1). It is known that mast cells and basophils are the only histamine-containing cells in humans (4). The lack of the difference between unpurified and purified mast cells according to their total histamine content is consistent with this fact.

The higher values in both spontaneous and stimulated histamine releases from unpurified mast cells than those from purified cells can be explained by a direct interaction between mast cells and nonmast cells (26).

Non-immunologic activation of human skin mast cells may be of great physiological importance. Calcium ionophore A23187, a widely-used representative of non-immunologic stimuli, complexes with calcium and can carry it across the membranes in both directions (29). Optimal concentrations of ionophore (1 μm) produces rapid releases of histamine in the presence of calcium (29,30). Additionaly, the effect of calcium ionophore is not affected significantly by the purification media (28). For these reasons, we preferred calcium ionophore A23187 as stimulating agent and obtained similar results consistent with previous studies (18,19,21).

Digestion of human neonatal foreskins by using an enzymatic dispersion and purification of these cells by density centrifugation have been presented to be a reproducible and efficient method for providing viable human skin mast cells. The method described in details above has several advantages for the studies on human skin mast cells. First of all, the tissue used in the experiments is disease-free and untreated with any kind of medication. Secondly, donors are from almost the same ages. Thirdly, the procedures are not complicated. Thus, this system is very practical for studying of mast cell responses to immunologic and non-immunologic stimuli.

It is expected that in the future, newly-developed in vitro and in vivo methods to investigate the mast cell functions and interactions will greatly increase the knowledge about the role and the importance of human cutaneous mast cells in biological and pathobiological conditions.

Acknowledgements

We thank the pediatric surgeons and the staff of Magee-Woman`s Hospital of Pittsburgh for provision of foreskin specimens.

REFERENCES

1. Longley J, Duffy TP, Kohn S. The mast cell and mast cell disease. J Am Acad Dermatol 1995;32:545-61.
2. Weber S, Krasagakes SK, Grabbe J, et al. Mast cells. Int J Dermatol 1995;34:1-10.
3. Church MK, El-Lati S, Okayama Y. Biological properties of human skin mast cells. Clin Exp Allergy 1991;21:1-9.
4. Goldstein SM, Witroub BU: The cellular and molecular biology of the human mast cell. In: Fitzpatrick TB, Eisen AZ, Wolf K, et al, eds. Dermatology in General Medicine: New York: McGraw-Hill, 1993:359-73.
5. Benyon RC. The human skin mast cell. Clin Exp Allergy 1989;19:375-87.
6. Galli SJ. New concepts about the mast cell. N Eng J Med 1993;328:257-65.
7. Kitamura Y. Heterogeneity of mast cells and phenotypic changes between subpopulations. Annu Rev Immunol 1989;7:59-76.
8. Lee TGD, Swieter M, Bienenstock J, et al. Heterogeneity in mast cell populations. Clin Immunol Reviews 1985;4:143-99.
9. Irani AA, Schechter NM, Craig LS, et al. Two types of human mast cell that have distinc neutral protease compositions. Proc Natl Acad Sci USA 1986;83:4464-8.
10. Schwartz L, Huff T. Biology of mast cells and basophils. In: Middleton E Jr, Reed CE, Ellis EF, et al, eds. Allergy, Principles and Practice: St.Louis: Mosby, 1993:135-68.
11. Schwartz LB. Heterogeneity of mast cells in human. In: Galli SJ, Austen KF, eds. Mast Cell and Basophil Differentitation and Function in Health and Disease: New York: Raven Press 1989:93-105.
12. Stevens RL, Austen KF. Recent advances in the cellular and molecular biology of mast cells. Immunol Today 1989;10:381-6.
13. Loveren HV, Teppema JS, Askenase PW. Skin mast cells. In: Bos JD, ed. Skin Immune System: Florida: CRC Press, 1990:171-93.

14. Bergstresser RP, Tigelaar RB, Tharp MD. Conjugated avidin identifies cutaneous rodent and human mast cells. J Invest Dermatol 1984;83:214-8.
15. Galli SJ. New insightes into "the riddle of the mast cells": microenvironmental regulation of mast cell development and phenotypic heterogeneity. Lab Invest 1990;62:5-33.
16. Holgate ST, Robinson C, Church MK:.Mediators of immediate hypersensitivity. In: Middleton E Jr, Reed CE, Ellis EF, et al, eds. Allergy, Principles and Practice: St.Louis: Mosby, 1993:267-301.
17. Tharp MD, Suvunrungsi RT, Sullivan TJ. IgE-mediated release of histamine from human cutaneous mast cells. J Immunol 1983;130:1896-901.
18. Benyon RC, Lowman MA, Church MK. Human skin mast cells: their dispersion, purification, and secretory characterization. J Immunol 1987;138:861-7.
19. Lawrence ID, Warner JA, Cohan VL, Hubbard WC. Purification and characterization of human skin mast cells. J Immunol 1987;139:3062-9.
20. Tharp MD, Sobotka AK, Fox CC, et al. Functional heterogeneity of human mast cell from different anatomical sites: in vitro responses to morphine sulfate. J Allergy Clin Immunol 1987;79:646-53.
21. Robinson C, Benyon RC, Holgate S, et al. The IgE- and calcium dependent release of eicosanoids and histamine from human purified cutaneous mast cells. J Invest Dermatol 1989;3:397-404.
22. Caulfield JP, El-Lati S, Thomas G, et al. Dissociated human foreskin mast cells degranulate in response to anti-IgE and substance P. Lab Invest 1990;63:502-10.
23. Siraganian RP. Histamine secretion from mast cells and basophils. TIPS 1983;4:432-7.
24. Morel AM, Delaage MA. Immunoanalysis of histamine through a novel chemical derivatization. J Allergy Clin Immunol 1988;82:646-54.
25. Coligan JE, Kruisbeek AM, Margulies DH. Trypan blue exclusion test of cell viability. In: Current Protocols in Immunology: New York: John Wiley & Sons, 1991:A.3.3.
26. Inagaki N, Kawasaki H, Ueno M, et al. Potentiation of antigen-induced histamine release from rat peritoneal mast cells through a direct interaction between mast cells and nonmast cells. Life Sciences 1994;54:1403-9.
27. Fewtrell C, Mohr C, Ryan TA, et al. Calcium: an important second messenger in mast cells. Ciba Foundation Symposium 1989;114-32.
28. Cabado AG, Vieytes MR, Botana LM. Rat pleural and peritoneal mast cells stimulated at different cellular levels: difference in and influence of purification media. Int Arch Allergy Appl Immunol 1993;100:234-9.
29. Diamant P, Patkar SA. Stimulation and inhibition of histamine release from isolated rat mast cells. Int Arch Allergy Appl Immunol 1975;49:183-207.
30. White JR, Pearce FL. Role of membrane bound-calcium in histamine secretion from rat peritoneal mast cells. Agent Actions 1981;11:324-9.

Correspondence: Mustafa ŞENOL, MD
İnönü Üniversitesi Tıp Fakültesi
Dermatoloji ABD
44300 MALATYA
Tel: 422-323 1753
Fax: 422-341 0036