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Isolation of Tissue Mast Cells

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ABSTRACT

Located primarily in tissues, mast cells are one of the principal effector cells in allergic inflammation. Mast cells derive from mononuclear precursor cells which undergo their final phase of differentiation in the tissues. Mast cells express a unique set of proteases and display functional diversity depending on the tissue in which they differentiate—a phenomenon often referred to as mast cell heterogeneity. Enzymatic digestion and density centrifugation have often been used to isolate human mast cells from tissues such as lung and skin, frequently resulting in cells with low viability and purity. Here, we describe a protocol that combines gentle enzymatic digestion with positive selection techniques to isolate reasonably viable and substantially enriched preparations of tissue mast cells. *Curr. Protoc. Immunol.* 90:7.25.1-7.25.11. © 2010 by John Wiley & Sons, Inc.

Keywords: mast cells • human mast cells • lung, skin • fluorescent activated cell sorting (FACS) • magnetic bead columns

INTRODUCTION

This unit describes methods for isolating and enriching mast cells from human skin or lung tissue. Basic Protocol 1 describes the isolation of mast cells from human skin and has been successfully used to isolate cells from breast tissue and human foreskin. Basic Protocol 2 details techniques for the isolation of mast cells from human lung tissue. The number and purity of mast cells obtained from the protocols described in this unit are sufficient for investigating mast cell receptor-induced mediator release, receptor-dependent chemotactic responses, proliferation, and survival. Genetic characterization using quantitative PCR or microarray analysis requires highly pure (>99%) mast cells and thus requires further enrichment using fluorescence-activated cells sorting or magnetic bead column isolations (also described here).

NOTE: Unless indicated, all tissue culture supplies and pipets are sterile disposable plasticware obtained from the following suppliers (also see *APPENDIX 5*): BD Falcon (tubes, cell strainers, tissue culture dishes and plates), Sarstedt (tissue culture flasks, bottle top filters), and Nunc (tissue culture flasks).

ISOLATION OF SKIN MAST CELLS

BASIC PROTOCOL 1

Mast cells are tissue-resident cells that are implicated in allergic inflammatory diseases such as asthma, atopic dermatitis, allergic rhinitis, and idiopathic urticaria. Upon cross-linking of their high-affinity IgE receptors (FcεRI), mast cells release preformed mediators (such as histamine, proteases tryptase, chymase, and TNF) and newly synthesized mediators (such as prostaglandins, leukotrienes, cytokines, chemokines, and growth factors). It is widely believed that skin mast cells are involved in a number of processes including the innate immune response to infectious organisms. In normal skin, mast cells, which are primarily of the tryptase/chymase subtype, occur in the greatest density in the superficial dermal zone (Church and Clough, 1999). Like all other types of

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mast cells, human skin mast cells bind IgE with high affinity to specific FcεRI receptors, but unlike those from lung or intestine, skin mast cells also express the C5a receptor (CD88) and degranulate in response to substance P, VIP, and compound 48/80 (Okayama et al., 1992). Some of the difficulties in studying mast cell biology are due to the difficulty in obtaining human mast cell lines that adequately mimic skin mast cells. The HMC-1 cell line does not generally express functional FcεRI receptors, does not contain stem cell factor, and is not stem cell factor dependent (Nilsson et al., 1994). Therefore, it is often necessary to isolate skin mast cells from fresh human tissue. Skin mast cells are typically isolated by mechanically and proteolytically digesting skin tissue. However, mast cells obtained from adult tissue using this method may be of limited purity (>50%) and suspect viability, and in a semi-activated state. The following protocols combine gentle enzymatic digestion with positive selection for FcεRI to optimally enrich skin mast cell populations.

CAUTION: When working with human tissue, cells, or infectious agents, biosafety practices should be followed.

Materials

Skin samples: fresh skin segments stored in sterile PBS (Invitrogen) at 4°C or on ice
Serum-free RPMI medium (see recipe for complete RPMI medium, but omit FBS)
Protease solution (see recipe)
PBS/DNase solution: 0.015 mg/ml bovine DNase I (Sigma-Aldrich, cat. no. DN25) in sterile PBS (Invitrogen)
Complete RPMI medium (see recipe)
Lysis buffer (see recipe)
RPMI/DNase solution: 0.015 mg/ml DNase I (Sigma-Aldrich, cat. no. DN25) in sterile serum-free RPMI (see recipe for complete RPMI medium, but omit FBS)
70% isotonic Percoll (with 30% RPMI/DNase; see recipe)
Recombinant human stem cell factor (Peprotech)

100× 15–mm petri dishes (Falcon)
50- and 15-ml conical graduated polypropylene centrifuge tubes (Falcon)
Tweezers, scissors, and scalpels, kept in sterile beaker with 70% ethanol
Tabletop centrifuge
Platform shaker
70-μm cell strainer (Falcon)

Additional reagents and equipment for counting cells using a hemacytometer (*APPENDIX 3A*), counting viable cells by trypan blue exclusion (*APPENDIX 3B*), and determining mast cell purity by toluidine blue staining (Support Protocol)

Prepare tissue fragments

1. Weigh skin samples (if under 1 g total tissue wet weight, do not use).

   Tissue segments that have been stored at 4°C longer than 24 hr should not be used. Tissue samples should never be frozen.

2. Place skin samples in a sterile petri dish and add 25 ml serum-free RPMI medium. Using sterile forceps, tweezers, and scissors, cut samples into small pieces (1 to 2 cm^3).

   It is important to keep the tissue samples wet at all times.

3. Using sterile tweezers, carefully place tissue pieces into a 50-ml conical tube and centrifuge 5 min at 200 × *g*, room temperature. Pipet off the medium and resuspend the tissue in 10 ml serum-free RPMI.

4. Centrifuge the tissue 5 min at 200 × *g*, room temperature.
5. Remove supernatant and split into as many 1- to 2-g samples (based on original wet weight) as necessary. Place each 1- to 2-g sample into a 50-ml conical tube.
6. Add 15 ml of protease solution to each tube of tissue and place tubes on a platform shaker at 37°C for 1 hr.
7. After 1 hr, remove supernatant and place in a new conical tube. Place supernatant on ice.
8. Add 15 ml of protease solution to tissue pellet and place back on platform shaker at 37°C for another 30 min.

Prepare cell suspension

9. Remove tissue from shaker and add 10 ml of PBS/DNase solution. Place back on the platform shaker for another 30 min.
10. Remove tissue from oven and filter through a 70-μm cell strainer to remove the large undigested pieces of tissue. Store filtrate on ice.
11. Rinse the tissue in the tube by shaking and swishing in fresh complete RPMI medium. Remove supernatant and filter through cell strainer. Discard the rest of the tissue, which at this point is mainly adipose tissue and may contain some oil droplets depending upon where the skin tissue was obtained.
12. Add complete RPMI to the filtrate up to 45 ml.

Isolate mast cells

13. Pool all supernatants from steps 8, 10, and 11 (the filtrate should not contain large pieces of tissue, but may contain red blood cells). To remove red blood cells, proceed to step 14. If the cell suspension does not contain many red blood cells, skip to step 17.
14. Resuspend each cell pellet by gently knocking the side of the tube. While gently mixing the tube by hand, add 3 ml of lysis buffer and incubate for 5 min at room temperature.
15. Add 10 ml of RPMI/DNase solution to stop the lysis reaction and gently mix the suspension by hand.
16. Centrifuge supernatants 10 min at 200 × *g*, room temperature. Resuspend cell pellet in 1 ml of RPMI/DNase medium.
17. In a sterile 15-ml conical tube, layer 1 ml of cell suspension onto 5 ml of 70% isotonic Percoll (with 30% RPMI/DNase) and centrifuge 20 min at 500 × *g*, 4°C.

    After spinning, the dense mast cells will have migrated through the Percoll and pelleted at the bottom of the tube.

18. Gently remove the first interface of the Percoll and discard. Resuspend the cell pellet 10 ml of complete RPMI and count viable cells using a hemacytometer (*APPENDIX 3A & APPENDIX 3B*).

    The viability of the mast cells can be determined by staining with trypan blue (APPENDIX 3B) and counting on a hemacytometer (APPENDIX 3A). Purity can be determined by making a cytospin and staining with toluidine blue (Support Protocol).

    Mast cell yield is highly variably and largely depends upon the type of skin tissue used (breast tissue has more mast cells than adult human skin) and the age of the donor (foreskin tissue from donor less than 2 years old has a greater density of mast cells). From 10 g of foreskin tissue from a >2 year old donor, for example, this procedure can yield ~1–3 × 10^5 cells. Mast cells obtained at the conclusion of this procedure are ~10% to 30% pure.

If greater purity is required, enrichment via positive selection column or fluorescence activated cells sorting can be used.

IMPORTANT NOTE: *Enrichment of mast cells using positive selection or cell sorting will result in decreased yields and viability.*

19. If desired, culture cells overnight in complete RPMI (10% FBS) supplemented with 10 ng/ml recombinant human stem cell factor.

ALTERNATE PROTOCOL 1

FLUORESCENCE-ACTIVATED CELL SORTING (FACS) OF SKIN MAST CELLS USING EXPRESSION OF FcεRI AND KIT

As an alternative to Percoll density centrifugation, skin mast cells can also be enriched by fluorescence activated cell sorting (FACS) using surface expression of FcεRI and Kit or positive selection using a column and magnetic beads conjugated to an antibody recognizing FcεRI (see Alternate Protocol 2).

***Additional Materials** (also see Basic Protocol 1)*

Phosphate-buffered saline (PBS; Invitrogen) containing 0.1% (w/v) bovine serum albumin (BSA), 4°C
Phosphate-buffered saline (PBS; Invitrogen)
Anti-FcεRI-APC (eBioscience)
Mouse IgG-APC (eBioscience)
Anti-CD117-PE (BD Pharmingen)
Mouse IgG_1-PE (BD Pharmingen)

5-ml polystyrene round-bottom tubes
Refrigerated centrifuge

Additional reagents and equipment for flow cytometry (Chapter 5)

1. Perform steps 1 to 16 of Basic Protocol 1. Use at least 1×10^5 cells should be per sample. Centrifuge 5 min at 200 × *g*, room temperature.
2. Resuspend cells with PBS containing 0.1% BSA (4°C) to a density of 1×10^6 cells/ml, then add 100 µl of the cell suspension per flow tube (5 ml polystyrene round-bottom tube).
3. Add anti-FcεRI-APC (or its isotype control; mouse IgG-APC) or anti-CD117-PE (or its isotype control; mouse IgG_1-PE) and incubate for 1 hr at 4°C in the dark.

   The optimal amount of antibody required is approximately 3 to 5 µg per 10^6 cells for each antibody, but antibody titration is recommended.
4. Wash off excess antibodies by adding 1 ml PBS containing 0.1% BSA to each tube and centrifuging 5 min at 200 × *g*, 4°C. Remove supernatant. Resuspend cell pellet in 100 µl fresh PBS/0.1% bovine serum albumin (BSA).
5. Sort $FcεRI^+/CD117^+$ cells using standard sorting procedures (Chapter 5). To preserve mast cell viability, ensure that the shear and flow rate of the sorter is low.

   Each sorter has different thresholds and sensitivities and it will be necessary to optimize the sorting settings.

   Cells obtained from FACS are generally not amenable to culture due to poor viability and susceptibility to contamination. However, if mast cells will be used for culture, ensure that the FACS machine is equipped with an aseptic sorting module and that all procedures are performed using sterile materials, including sterile sheath fluid.

ENRICHMENT OF SKIN MAST CELLS BY POSITIVE SELECTION IMMUNOMAGNETIC SEPARATION

ALTERNATE PROTOCOL 2

This protocol describes a relatively simple immunomagnetic purification procedure that isolates mast cells using positive selection.

***Additional Materials** (also see Basic Protocol 1)*

Phosphate-buffered saline (PBS; Invitrogen) containing 0.1% (w/v) bovine serum albumin (BSA), 4°C
Phosphate-buffered saline (PBS; Invitrogen)
Anti-PE MicroBeads and wash buffer (Miltenyi Biotec)
Anti-FcεRI- APC (eBioscience)
Mouse IgG-APC (eBioscience)
Anti-CD117-PE (BD Pharmingen)
Mouse IgG_1-PE (BD Pharmingen)
MACS column and magnet (Miltenyi Biotec)

Additional reagents and equipment for trypan blue exclusion test for cell viability (*APPENDIX 3B*) and toluidine blue staining for mast cell purity (Support Protocol)

Prepare and label cells

1. Perform steps 1 to 16 of Basic Protocol 1. Use at least 1×10^5 cells should be per sample. Centrifuge 5 min at 200 × *g*, room temperature.
2. Resuspend cells with PBS containing 0.1% BSA (4°C) to a density of 1×10^6 cells/ml. Separate into 600-µl aliquots in microcentrifuge tubes.
3. Label cells by adding anti-FcεRI-PE antibody to a final concentration of 5 µg/10^6 cells. Mix well and incubate at 4°C for 30 min.
4. Wash off excess antibodies by adding 1 ml PBS containing 0.1% BSA to each tube and centrifuge for 5 min at 200 × *g*, 4°C. Remove supernatant and resuspend cell pellet in 80 µl fresh PBS containing 0.1% BSA.
5. Add 20 µl of anti-PE MicroBeads and mix well. Incubate for 30 min at 4°C.
6. Wash cells by adding 1 ml of PBS containing 0.1% BSA to each tube and centrifuge 5 min at 200 × *g*, 4°C. Aspirate supernatant completely and resuspend in 500 µl of PBS containing 0.1% BSA.

Perform magnetic separation

7. Prepare magnetic column and place in magnetic separator according to the manufacturer's instructions.
8. Apply cell suspension onto the column and collect unlabeled cells that pass through the column. Wash column with PBS containing 0.1% BSA and collect effluent.

   This is the fraction that contains unlabeled, contaminating cells.

9. Remove the column from the separator and place on a suitable collection tube. Immediately flush the column with PBS containing 0.1% BSA and firmly push the plunger into the column.
10. Collect the effluent; this is the fraction that contains the PE-labeled mast cells. Determine purity by toluidine blue staining (see Support Protocol) and viability with trypan blue staining (*APPENDIX 3B*).

BASIC PROTOCOL 2

ISOLATION OF HUMAN LUNG MAST CELLS

The procedure for isolating lung mast cells is similar to the procedure for isolating skin mast cells, with a few notable exceptions. Tyrode's buffer is used in the initial stages of the isolation. The protease solution used to disperse tissues contains only collagenases and requires shorter incubation times for digestion of connective tissue. Like skin mast cells, human lung mast cells bind IgE with high affinity to specific FcεRI receptors, but, unlike skin mast cells, lung mast cells do not express the C5a receptor (CD88) and are not activated by cationic molecules such as substance P, VIP, and compound 48/80. IgE-dependent stimulation by activating tyrosine kinases induces characteristic compound exocytosis resulting in the liberation of the preformed mediators and production of prostaglandin D2 and leukotriene C4. In general, isolation of mast cells from human lung tissue is faster and more straightforward and requires the use of only one digestive enzyme: collagenase.

Mast cell yield is highly variable and the best yields are obtained from fresh and healthy lung tissue (from nonsmokers). From 10 g of healthy lung tissue, this procedure can yield ~1–3 × 10^6 mast cells. Mast cells obtained at the conclusion of this procedure are approximately 10% to 30% pure. If greater purity is required, follow the protocols for enrichment using flow cytometry (see Alternate Protocol 1) or immunomagnetic separation (see Alternate Protocol 2).

Materials

Fresh lung tissue stored in sterile PBS at 4°C or on ice
Modified Tyrode's buffer (see recipe)
0.5 mg/ml collagenase in sterile PBS
PBS/DNase solution: 0.015 mg/ml bovine DNase I (Sigma-Aldrich) in sterile PBS (Invitrogen)
Lysis buffer (see recipe)
RPMI/DNase solution: 0.015 mg/ml bovine DNase I (Sigma-Aldrich) in serum-free RPMI medium (see recipe for complete RPMI medium, but omit FBS)
70% isotonic Percoll (with 30% RPMI/DNase see recipe)
Complete RPMI medium (see recipe)
Recombinant human stem cell factor (Peprotech)

100 × 15–mm petri dishes (Falcon)
Tweezers, scissors, and scalpels, kept in sterile beaker with 70% ethanol
50- and 15-ml conical graduated polypropylene centrifuge tubes (Falcon)
Platform shaker
70-μm cell strainer

Additional reagents and equipment for counting cells using a hemacytometer (*APPENDIX 3A*), counting viable cells by trypan blue exclusion (*APPENDIX 3B*), and determining mast cell purity by toluidine blue staining (Support Protocol)

Prepare tissue fragments

1. Weigh lung samples (if under 5 g total tissue wet weight, do not use).

   Tissue segments that have been stored at 4°C longer than 24 hr should not be used. Tissue samples should never be frozen.

2. Place lung samples in a sterile petri dish and add 25 ml modified Tyrode's buffer. Using sterile forceps, tweezers, and scissors, cut samples into small pieces (1 to 2 cm^3).

   It is important to keep the tissue samples wet at all times.

3. Using sterile tweezers, carefully place tissue pieces into a 50-ml conical tube and centrifuge 5 min at 200 × *g*, room temperature. Pipet off the medium and resuspend the tissue in 10 ml Tyrode's buffer.
4. Centrifuge the tissue 5 min at 200 × *g*, room temperature.
5. Remove supernatant and split into as many 1- to 2-g samples (based on original wet weight) as necessary. Place each 1- to 2-g sample into a 50-ml conical tube.
6. Add 15 ml of 0.5 mg/ml collagenase (in PBS) to each tube of tissue and place tubes on a platform shaker at 37°C for 15 min.
7. After 15 min, remove supernatant and place in a new conical tube. Place supernatant on ice.
8. Add 15 ml of 0.5 mg/ml collagenase (in PBS) to tissue pellet and place back on platform shaker at 37°C for another 30 min.

Prepare cell suspension

9. Remove tissue from platform shaker and add 10 ml of PBS/DNase solution. Place back onto platform shaker for another 30 min.
10. Remove tissue from 37°C incubator and filter through a 70-μm cell strainer to remove the large undigested pieces of tissue. Store filtrate on ice.
11. Rinse the tissue in the tube by shaking and swishing in fresh modified Tyrode's buffer. Remove supernatant and filter through cell strainer.
12. Add modified Tyrode's buffer to the filtrate up to 45 ml.

Isolate mast cells

13. Pool all supernatants from steps 8, 10, and 11 (the filtrate should not contain large pieces of tissue, but may contain red blood cells). To remove red blood cells proceed to step 14. If the cell suspension does not contain many red blood cells, skip to step 17.
14. Resuspend each cell pellet by gently knocking the side of the tube. While gently mixing the tube by hand, add 3 ml of lysis buffer and incubate for 5 min at room temperature.
15. Add 10 ml of PBS/DNase solution to stop the lysis reaction and gently mix the suspension by hand.
16. Centrifuge 10 min at 200 × *g*, 4°C. Resuspend cell pellet in 1 ml of RPMI/DNase.
17. In a sterile 15-ml conical tube, layer 1 ml of cell suspension onto 5 ml of 70% isotonic Percoll containing 30% RPMI/DNase, and centrifuge 20 min at 200 × *g*, 4°C.

    After spinning, the dense mast cells will have migrated through the Percoll and pelleted at the bottom of the tube.

18. Gently remove the first interface of the Percoll and discard. Resuspend the cell pellet 10 ml of complete RPMI and count viable cells using a hemacytometer (*APPENDIX 3A & APPENDIX 3B*).

    The viability of the mast cells can be determined by staining with trypan blue (APPENDIX 3B) and counting on a hemacytometer. Purity can be determined by making a cytospin and staining with toluidine blue (Support Protocol).

19. If desired, culture cells overnight in complete RPMI (10% FBS) supplemented with 10 ng/ml recombinant human stem cell factor.

SUPPORT PROTOCOL

ACID TOLUIDINE BLUE STAINING OF MAST CELLS

Both connective tissue and mucosal mast cells contain granules within their cytoplasm that exhibit blue metachromasia upon fixation of the mast cells in Mota's fixative followed by staining with acid toluidine blue. The internal structure of mast cells is best visualized when slide preparations are made using a cytocentrifuge.

Materials

Mota's fixative (see recipe)
66% and 100% ethanol
Acid toluidine blue solution (see recipe)

Cytocentrifuge (Cytospin, Shandon/Lipshaw)
Microscope slides

1. Cytocentrifuge cells onto a microscope slide according to the manufacturer's instructions.
2. Flood slide with Mota's fixative and let sit for 10 min.
3. Flood slide with 66% ethanol to remove fixative.
4. Wash slide by dipping it into a beaker of distilled water.
5. Flood slide with acid toluidine solution and let sit for 10 min. Repeat washing as in step 4.
6. Flood slide with 66% ethanol and repeat washing as in step 4.
7. Flood slide with 100% ethanol. Repeat washing as in step 4 and allow slide to air dry.

REAGENTS AND SOLUTIONS

Use deionized, distilled water in all recipes and protocol steps. For common stock solutions, see ***APPENDIX 2A****; for suppliers, see* ***APPENDIX 5****.*

Lysis buffer

1.37 g NH_4Cl
0.515 g Tris base
250 ml H_2O
Adjust pH to 7.2 with 1 M NaOH or HCl
Store up to 6 months at 4°C

Mota's fixative

25 ml distilled H_2O
2 g lead acetate, basic (Sigma-Aldrich, cat. no. 32306)
1 ml glacial acetic acid
25 ml 100% ethanol

To a 100-ml beaker, add the distilled water and the lead acetate. While stirring, add the glacial acetic acid dropwise to help the lead acetate dissolve. Add ethanol at the end, after the lead acetate has dissolved.

This solution can be stored up to 2 to 4 months at room temperature.

Percoll, isotonic, 70%, plus 30% RPMI/DNase

Prepare isotonic Percoll:
7 ml Percoll (Sigma-Aldrich), sterile
1 ml 10× Hanks' balanced salt solution (HBSS, e.g., Invitrogen), sterile

continued

0.1 ml fetal bovine serum (FBS), sterile
1.9 ml double-distilled H_2O, sterile

Under sterile conditions, add all reagents to a 15-ml conical tube, vortex, and use immediately. Always prepare fresh on day of cell isolation and discard unused solution.

Prepare RPMI/DNase:
0.015 mg/ml DNase in complete RPMI (see recipe)

Prepare working Percoll solution (70% isotonic Percoll/30% RPMI/DNase):
Mix 7 parts isotonic Percoll with 3 parts RPMI/DNase (prepare fresh).

Protease solution

RPMI (e.g., Invitrogen) containing:
0.2 mg/ml collagenase (Sigma-Aldrich)
0.1 mg/ml hyaluronidase (Sigma-Aldrich)
0.2 mg/ml protease (Sigma-Aldrich)
Prepare fresh on day of cell isolation; discard unused solution

Place in 37°C water bath to dissolve.

RPMI medium, complete

RPMI medium containing L-glutamine and sodium bicarbonate (Invitrogen) supplemented with:
10% fetal bovine serum (FBS; Invitrogen)
100 U/ml penicillin/100 μg/ml streptomycin (add from 100× pen-strep; e.g., Invitrogen)
0.25 M HEPES (add from 1 M stock)

Filter sterilize through a 0.2 μm bottle-top filter. Adjust pH to 7.6.

Supplemented RPMI medium can be stored for 3 to 4 weeks at 4°C in the dark.

Toluidine blue solution, acid

35 ml distilled H_2O
15 ml 100% ethanol
1 to 2 ml concentrated HCl
0.25 g toluidine blue powder (Sigma-Aldrich, cat. no. 89640)

To a 50-ml bottle, add the distilled water and ethanol. Place the solution on a pH meter and add HCl dropwise until the pH of the solution is ∼1 (1.5 is acceptable). Remove solution from pH meter (clean electrode with water) and add the toluidine blue powder to the solution. Mix by stirring on a stir plate until dissolved. Observe proper precautions for handling acidic solutions.

This solution can be stored indefinitely at room temperature.

Tyrode's buffer, modified

137 mM NaCl
2.8 mM KCl
12 mM $NaHCO_3$
5.5 mM glucose
0.4 mM NaH_2PO_4
10 mM HEPES, pH 7.4
3.5 mg/ml BSA
Store up to 6 at 4°C

COMMENTARY

Background Information

Mast cells develop from progenitor cells that arise from uncommitted hematopoietic stem cells in the bone marrow. These progenitors express the stem cell factor receptor (SCF receptor or Kit) and represent the major survival and differentiation pathway for mast cells. The interactions between mast cells and the tissue in which they differentiate are crucial to their differentiation and maturation. Mast cells are also distributed in the deeper regions of the central nervous system, the upper and lower respiratory epithelium, the bronchial lumen, the gastrointestinal mucosa and submucosa, bone marrow, and skin. In the skin, lungs, and gastrointestinal tract, mast cell concentrations approximate 10,000 to 20,000 cells/mm^3, and these tissues are therefore suitable sources of mast cells for research (Mikhail and Miller-Milinska, 1964). Connective tissue cells, including fibroblasts, contribute to differentiation and maturation of mast cells in tissue by releasing SCF, nerve growth factor (NGF), adhesion molecules, and other as yet uncharacterized growth factors. Two mast cell subtypes have been described in tissue—the mucosal (MC_T) or connective tissue (MC_{TC}) mast cells. These subtypes are based on structural, biochemical, and functional differences, but it is becoming increasingly clear that connective-type cells can be found at mucosal surfaces and vice versa. The terms "connective tissue mast cell" and "mucosal mast cell" were first described in rodent models where mast cells follow a different differentiation pathway dependent largely upon IL-3. However, in the rodent system, the protease content of connective-tissue versus mucosal mast cells is different and a bit more complex. In the human, distinctive features have been used to differentiate these two subsets. The MC_T mast cell predominantly expresses the protease tryptase and mast cells isolated from mucosal surfaces such as the lung tend to be rich in MC_T. Furthermore, MC_T are present in elevated numbers in the mucosa of patients suffering from allergic inflammation or parasitic disease (Bischoff, 2009). Structurally, these cells have distinctive scroll granules and are often found in close proximity to T cells (Mekori and Metcalfe, 1999). The MC_{TC} mast cells express tryptase, chymase, carboxypeptidase, and cathepsin G, and predominate in the gastrointestinal tract as well as the skin, synovium, and subcutaneous tissue (Miller and Pemberton, 2002). Patients with fibrotic diseases often show an increase in MC_{TC} in the affected tissues, whereas patients with allergic disease do not show these types of changes (Reid et al., 2007).

Mast cells generated from precursor CD34+ in vitro or human mast cells lines such as HMC-1 and LAD2 are excellent models of mast cell biology in that they provide large and pure populations of homogenous mast cells (Kirshenbaum et al., 1999; Kirshenbaum, 2000). However, these mast cells are sometimes immature in phenotype (HMC-1) and are grown in conditions that do not adequately model their tissue environment in vivo. As such, it is often necessary to isolate mast cells from human tissue. Mast cells are large (10 to 15 μm in diameter) and possess numerous densely packed granules (Dvorak et al., 1993). The protocols described in this unit take advantage of mast cell size and density and their expression of FcεRI and Kit to generate enriched populations of either MC_T or MC_{TC}. The gastrointestinal tract is another rich source of human mast cells, and protocols for their isolation have been described (Sellge and Bischoff, 2006).

Critical Parameters and Troubleshooting

Isolation of tissue mast cells is variable and challenging, and a successful isolation depends largely on fresh, healthy tissue from younger donors. Loss of cells can occur during Percoll density centrifugation, since an incorrectly constituted protocol will change the density of the medium and disrupt the ordered sedimentation of the mast cells. If a pellet does not form after centrifugation, make sure that the correct centrifugal force was used and that the Percoll was mixed correctly. If mast cells are cultured after isolation, contamination with fungi and bacteria is often a problem. The sterility of the starting human tissue is often difficult to control, but, whenever possible, only sterile solutions should be used, and all work should be done in a properly functioning biosafety hood. Should contamination occur, immediately bleach and discard all contaminated flasks and scrub and clean all surfaces that have contacted these flasks with 70% ethanol.

Anticipated Results

Generally, mast cell isolation from lung is faster and produces higher numbers of mast cells with reasonable purity. However, mast cell yield is highly variable, and depends

largely on the health, age, and freshness of the tissue samples used.

From 10 g of foreskin tissue from a >2 year old donor, the procedure described in this unit can yield ~1–3 × 10^5 cells. From 10 g of healthy lung tissue, the procedure in this unit can yield ~1–3 × 10^6 mast cells. Mast cells isolated from either lung or skin after Percoll centrifugation, but prior to flow cytometric enrichment, are ~10% to 30% pure.

Time Considerations

The initial dissociation and enzymatic digestion of human tissue and Percoll centrifugation can take ~6 to 8 hr, depending upon the amount of starting tissue.

Mast cell enrichment by flow cytometry can take 5 to 6 hr depending on the set-up time for the sorter (which can take 2 to 3 hr to flush the system for aseptic sorting) and the sort speed.

Mast cell enrichment by magnetic column can take approximately 2 to 3 hr.

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