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Measuring Mast Cell Mediator Release

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Abstract

Mediators released from activated mast cells are responsible for the allergic inflammatory reactions associated with disease states such as anaphylaxis and atopy. These mediators are released as a consequence of immediate degranulation and phospholipid metabolism, upon mast cell activation, followed by delayed cytokine gene expression. Thus, techniques that monitor indices of these events in mast cell culture systems, in association with biochemical analysis of parameters of cell signaling, are critical to our understanding of the molecular mechanisms regulating mast cell-mediated disease. Furthermore, these systems can be adapted for high throughput screens to identify potential inhibitors of mast cell activation which may provide potential leads for novel therapies for these diseases. In this unit, we describe approaches which can be readily used or adapted for a variety of rodent and human mast cell culture systems for the determination of degranulation, phospholipid-derived inflammatory mediator production, and cytokine generation.

Key Terms

cytokines; degranulation; eicosanoids; FcεRI; human mast cells (HuMCs); KIT; mouse bone marrow-derived mast cells (BMMCs); stem cell factor (SCF)

Measuring mast cell mediator release

This unit presents protocols for measuring mediator release from both rodent and human mast cell cultures. These protocols can be used in conjunction with those described for the generation and isolation of rodent mast cells and mast cell lines (Unit 3.23) and human mast cells and mast cell lines ("Generation, Isolation, and Maintenance of Human Mast Cells and Mast Cell lines" Unit.) This unit is divided into three sections. The first section describes protocols designed to monitor mast cell degranulation through the measurement of the release of the granule component, β -hexosaminidase; the second section describes protocols for determining the generation of products of phospholipid metabolism, the eicosanoids, leukotriene C₄ (LTC₄) and prostaglandin D₂ (PGD₂); and the third section describes protocols for determining the generation of multiple cytokines.

Basic Protocol 1

Measurement of mast cell degranulation as monitored by β -hexosaminidase release

Materials

Cells: Mouse bone marrow-derived mast cells (BMMCs), primary human mast cells (HuMCs) obtained from CD34⁺ peripheral blood progenitor cells, or the human LAD2 mast cell line.

Media for BMMCs: RPMI1640 supplemented with 10% FBS, glutamine (4 mM), sodium pyruvate (1 mM), penicillin (100 units/ml), streptomycin (100 μ g/ml), non-essential amino acids (10 ml/L) (Sigma-Aldrich), HEPES (25 mM), β -mercaptoethanol (50 μ M), and mouse recombinant IL-3 (30 ng/ml) (Peprotech).

Media for HuMCs: StemPro-34 culture media (GIBCO) containing the StemPro-34 nutrient supplement as indicated by the manufacturer (GIBCO), glutamine (2 mM), penicillin (100 units/ml), streptomycin (100 μ g/ml), recombinant human IL-3 (30 ng/ml) (for use in first week cultures only), IL-6 (100 ng/ml), and human stem cell factor (SCF) (100 ng/ml) (Peprotech)

Media for LAD2 cells: StemPro-34 culture media (GIBCO) containing the StemPro-34 nutrient supplement as indicated by the manufacturer (GIBCO), glutamine (2 mM), penicillin (100 units/ml), streptomycin (100 μ g/ml), human SCF (100 ng/ml) (Peprotech)

Mouse monoclonal anti-DNP-IgE (clone SPE-7) (Sigma-Aldrich)

Dinitrophenyl-human serum albumin conjugate (DNP-HSA) (Sigma-Aldrich)

Human myeloma IgE (Calbiochem), biotinylated using NHS-biotin (Zymed) (see Unit 5.3. support protocol 2)

Streptavidin (Sigma-Aldrich)

HEPES buffer (pH 7.4): HEPES (10 mM), NaCl (137 mM), KCl (2.7 mM), Na₂HPO₄·7H₂O (0.4 mM), glucose (5.6 mM), CaCl₂·2H₂O (1.8mM), MgSO₄·7H₂O (1.3mM), bovine serum albumin (BSA) (0.04%)

Citrate buffer (pH 4.5): citric acid (40 mM), Na₂HPO₄·7H₂O (20 mM)

Glycine (400 mM; pH 10.7)

Triton X-100 (0.1%)

p-nitrophenyl N-acetyl- β -D-glucosamide (PNAG) (Sigma-Aldrich)

96 well plates

15 ml polypropylene conical tubes

1.5 ml e-tube

Sonicator (e.g. Cole Parmer Ultrasonic Homogenizer)

8 or 12 tip multichannel pipettor

Table top centrifuge (e.g. Sorvall RT-6000B refrigerated centrifuge)

NOTE: All solutions are made in distilled water. All solutions and media must be filtered and kept in cold room.

Procedures

1. BMMCs are obtained by flushing bone marrow cells from the femurs of C57BL/6J mice (The Jackson Laboratory), then culturing the cells for 4–6 weeks in RPMI containing mouse recombinant IL-3 (30 ng/ml) (see Unit 3.23). BMMCs can also be obtained from other mouse strains by this protocol. BMMCs are generally harvested for use between 4–6 weeks of culture.

Primary HuMCs are developed from CD34⁺-peripheral blood progenitor cells in StemPro-34 culture media containing recombinant human IL-3 (first week cultures only), IL-6, and stem cell factor and are used between 7–10 wk after initiation of culture. LAD2 cells are cultured in StemPro-34 culture media containing human SCF (100 ng/ml). (“Generation, Isolation, and Maintenance of Human Mast Cells and Mast Cell lines” Unit)

Cells are sensitized overnight with an optimal concentration (100 ng/ml) of biotinylated human IgE (for HuMCs or LAD2 cells) or mouse anti-DNP-IgE (for BMMCs).

NOTE: In some cases, BMMCs are cultured in SCF and IL-3 in RPMI. In this case, the results may be different from the cells cultured in IL-3 only.

NOTE: The genetic background of the mice influences the extent of FcεRI-mediated mast cell mediator release. For this reason it is essential to use the same genetic background, ideally littermate controls, of knock out and wild type for direct comparisons.

NOTE: All incubations are performed in a humidified 37 °C, 5% CO₂ incubator unless otherwise specified.

NOTE: To avoid the influence of IL-3, or where used SCF, on results, cells should be starved from 4 h to overnight, in cytokine-free medium.

NOTE: Prepare adequate numbers of cells for duplicate assays and to anticipate procedural losses.

NOTE: HuMCs, LAD2 and BMMCs are non-adherent cells. To replace medium or wash the cells, spin the cells at 450 x g, room temperature (RT) for 5 min and carefully remove the supernatant; treat the cells gently.

2. Following sensitization, wash the cells three times with 10 ml HEPES buffer (maintained at 37 °C) to remove excess IgE.
3. Resuspend cells in the HEPES buffer and aliquot the cells into 96 well plates (HuMCs, LAD2 cells 5,000–10,000/well, BMMCs 30,000–50,000/well). Final volume per well is 100 µl including agonist. As agonists and antagonists are added in 10 µl of 10× final concentrations, cell suspensions are adjusted to accommodate this volume. For example, if the cells are treated with one agonist, the cells are adjusted such that the correct final amount of cells is added in 90 µl. If the cells are challenged with one agonist and one antagonist, then the cells are added in 80 µl.

NOTE: Samples must be run at least in duplicate. To correct for background fluorescence, prepare blanks (HEPES buffer only).

4. Incubate cells in 37 °C for 5–10 minutes to equilibrate the cells with activation temperature.

5. Prepare 10× stocks of agonists or inhibitors in HEPES buffer. Then, add 10 µl of these stocks to each well. For example, to stimulate BMMCs with 100 ng/ml of DNP-HSA, prepare 1 µg/ml of DNP-HSA in HEPES buffer and add 10 µl into 90 µl of the cell suspension in the well.

NOTE: For inhibitor studies, cells are preincubated with inhibitors for 10–30 min prior to the addition of the agonists. When using SCF or other agonist such as G-protein coupled receptor (GPCR) agonist, add just prior to, or concurrently with antigen.

6. Incubate the plate in a heating block or warm air oven at 37 °C for 30 min (without CO₂).
7. During incubation, prepare p-nitrophenyl N-acetyl-β-D-glucosamide (PNAG) solution in citrated buffer.

NOTE: Calculate the total volume required based on a volume of 10 ml per complete 96 well plate. Dissolve 35 mg of PNAG per 10 ml of citrate buffer. PNAG is poorly soluble in the citrate buffer, therefore sonication may be required to fully solubilize.

8. Aliquot 100 µl of PNAG solution into two new 96 well plates using a multichannel pipettor. One plate is for measurement of secreted β-hexosaminidase activity (supernatants) and the other is used to calculate the total amount (supernatants and lysates) to determine the percentage release.
9. After step 6, spin the plate 450 × g, at 4 °C for 5 min to stop the reaction and to ensure the cells are sedimented to the base of the wells.
10. Tilt the plate to an angle of 45° then CAREFULLY remove 50 µl aliquots of the cell free supernatant using a multichannel pipettor by removing from the top of where the depth of the solution is greatest. Add the aliquots into the PNAG solution and incubate the plate for 90 min at 37 °C (without CO₂).

NOTE: Make sure the cells are not removed then transferred into PNAG solution when you take 50 µl of supernatant as this will produce an artificially high degranulation value.

11. In order to calculate the total β-hexosaminidase activities, add 150 µl of 0.1% Triton X-100 solution into the 50 µl of supernatants and cells remaining in the original incubation plate. Resuspend carefully and take 50 µl of lysates and add into the PNAG solution and incubate the plate for 90 min at 37 °C (without CO₂). Alternatively, add 150 µl of distilled water instead of 0.1% Triton X-100 solution and freeze the plate for over 1 hour at –80 °C. Defrost, mix by pipetting up and down then remove 50 µl to assess lysate content as above..
12. After incubation, add 50 µl 0.4 M Glycine buffer into each well. The appearance of yellow color indicates the extent of β-hexosaminidase activity.
13. Read plate absorbance at 405 nm with reference filter at 620 nm. Calculate the percentage of β-hexosaminidase activities present in the supernatants.

Calculations

- i.
$$\text{Percentage degranulation} = 100 \times (\text{superatant content}) / (\text{supernatant} + \text{lysate content})$$
- ii. However only ½ of supernatant is assayed and plate blank needs to be deducted.

Therefore numerator (total supernatant reading) = $2 \times (\text{supernatant} - \text{plate blank})$

- iii. Only $\frac{1}{4}$ of lysates is used and lysates still contain $\frac{1}{2}$ of supernatant. Plate blank also needs to be deducted.

Therefore denominator = (total cell content prior to stimulation)

$$= (1/2 (\text{total supernatant} - \text{plate blank})) + (4 \times (\text{lysates} - \text{plate blank}))$$

- iv.

$$\% \text{ release therefore} = 100 \times (2 \times (\text{supernatant} - \text{plate blank})) / (1/2 (\text{total supernatant} - \text{plate blank})) + (4 \times (\text{lysates} - \text{plate blank}))$$

For example if supernatant reading = 0.4, lysates = 0.3, and plate blank = 0.04

$$\text{Then } \% \text{ release} = 100 \times (2 \times (0.4 - 0.04)) / ((0.4 - 0.04) + (4 \times (0.3 - 0.04))) = 51 \% \text{ release}$$

These calculations can readily be carried out using a spread sheet such as MS-Excel.

Measurement of eicosanoid (PGD₂, LTC₄) generation in mast cells

Materials—Cells (BMMCs, HuMCs), media, and other materials are the same as in “Measurement of degranulation as monitored by β -hexosaminidase release from mast cells”

Prostaglandin D₂-MOX EIA kit (e.g., Cayman chemical)

Leukotriene C₄ EIA kit (e.g., Cayman chemical)

NOTE: PGD₂ is chemically unstable so it can rapidly degrade in the presence of serum. To avoid degradation a PGD₂ Methoxime enzyme immunoassay (PGD₂-MOX EIA) is used which is based on the conversion of PGD₂ to its stable MOX derivate.

NOTE: Prepare ultra pure water for the eicosanoid measurement assay. Water must be deionized and free of trace organic contaminations. To accomplish the latter use an activated carbon filter cartridge or organic scavengers.

1. HuMCs or BMMCs are sensitized overnight with an optimal concentration (100 ng/ml) of biotinylated human IgE or mouse SPE-7, respectively, in cytokine and stem cell factor-free medium.

NOTE: All incubations are performed in a humidified 37°C, 5% CO₂ incubator.

NOTE: Assays are performed in duplicate so prepare an appropriate number of cells to account for this and also for procedural loss.

2. The next day, wash the cells three times with 10 ml warm HEPES buffer to remove excess IgE.
3. Resuspend cells with HEPES buffer and aliquot the cells into 96 well plates or 1.5 ml e-tube (HuMCs; 200–250 cells/sample for both PGD₂ and LTC₄, BMMCs;

2000 cells/sample for PGD₂, 100 cells/sample for LTC₄). Final volume will be 100 µl.

NOTE: Cell number is very critical for measurement of antigen-induced eicosanoid generation. In the case of LTC₄ measurement in BMMCs, only 100 cells are needed. This low number can easily lead to inaccuracies in cell quantification. Therefore, it is recommended to use 100–1000 fold more cells and dilute the supernatants later with HEPES buffer.

NOTE: To measure the generation of eicosanoids in response to other agonists such as SCF, GPCR agonist, etc, it may be necessary to first determine the optimal cell number.

4. Incubate cells at 37 °C for 5–10 minutes to equilibrate the cells with the activation temperature then add 1/10 of the final reaction volumes of 10× stocks of antigen in HEPES buffer. (DNP-HSA for BMMCs, Streptavidin for HuMCs (10–100 ng/ml is the optimal concentration range).
5. Incubate the plate at 37 °C for 20–30 min.
6. Spin down the plate (450 × g, 5 min, 4 °C) or 1.5 ml e-tube to recover free supernatant. Store the supernatants at –80 °C until assayed.

NOTE: Eicosanoids are chemically unstable and can rapidly degrade. Samples should thus be assayed immediately after collection or stored at –80 °C until assayed.

7. Measure LTC₄ and PGD₂ in the supernatants by competitive enzyme immunoassay (Cayman Chemicals) according to the manufacturer's instructions. The amounts of LTC₄ and PGD₂ are calculated based on the standard curve (10 pg/ml–1000 pg/ml).

NOTE: A new standard curve must be run for each assay as results can vary depending on the development condition.

Measurement of cytokine release from mast cells

Materials—Cells (BMMCs, HuMCs), media, and other materials are the same as for "Measurement of degranulation as monitored by β-hexosaminidase release from mast cells".

24 well plate or 48 well plate

Cytokine assay kit (e.g., R&D systems)

NOTE: All incubations are performed in a humidified 37°C, 5% CO₂ incubator.

1. BMMCs are starved of cytokines and sensitized overnight with an optimal concentration (100 ng/ml) of mouse anti-DNP IgE (SPE-7) as for degranulation.

HuMC are sensitized overnight with (100 ng/ml) of biotinylated human IgE in complete medium (containing SCF and IL-6).

NOTE: In the case of HuMCs, antigen or SCF alone fails to generate sufficient cytokine to be detected. To detect cytokine release, it is necessary to incubate the cells with antigen and SCF together.

2. The next day wash BMMCs or HuMCs three times with 10 ml warm cytokine free medium or complete medium respectively, to remove excess IgE.

3. Resuspend BMMCs with cytokine-free medium and aliquot the cells into a 24 or 48 well plate ($0.5\text{--}1 \times 10^6$ cells/ml). For HuMCs, resuspend the cells with complete medium and aliquot the cells into a 24–48 well plate (1×10^6 cells/ml). For measurement of cytokines, 100 μ l/sample is used.

NOTE: Perform cytokine measurement at least in duplicate (100 μ l/sample). Therefore prepare enough cells.

4. Prepare 10 $\times$ stocks of antigen in the same media. (DNP-HSA for BMMCs, Streptavidin for HuMCs). For BMMCs and HuMCs, 10–100 ng/ml antigen concentration is optimal.

NOTE: HuMC media contains 100 ng/ml of SCF, therefore it is not necessary to add additional SCF.

5. Incubate the cells 37 °C for 4–8 hours.

NOTE: In mast cells, antigen/SCF stimulation increases the mRNA and protein synthesis for multiple cytokines. Although it depends on the cytokine generated, generally 2–4 hours is the optimal time for detecting mRNA message and 4–8 hours is optimal for protein detection. For each new cytokine, it will be necessary to optimize the incubation time.

6. Pipette the supernatants into 1.5 ml e-tube and spin the cells ($5000 \times g$ for 2 min) to prepare cell-free supernatant. Store the supernatants at -80 °C until assayed.

7. Measure the cytokine content according to the manufacturer's instructions. The amount of cytokine or chemokine is calculated based on the standard curve (32.5 pg/ml–2000 pg/ml).

NOTE: If the samples' OD is above the standard curve range, the samples will need to be diluted to 1/5 or 1/10.

COMMENTARY

Background

Mast cell mediators likely evolved to participate in innate and adaptive defense mechanisms designed to protect the body against invading pathogens and parasites (Brown et al. 2008, Marshall 2004). However these mediators have the capacity to produce potentially life-threatening or otherwise disabling consequences in those within the general population susceptible to anaphylactic reactions and atopy (Brown et al. 2008, Metcalfe et al. 1997). These mediators are released upon mast cell activation following antigen-induced aggregation of the high affinity receptors for IgE (Fc ϵ RI) following binding of the antigen to antigen-specific IgE occupying these receptors (Gilfillan and Tkaczyk 2006). Multiple other receptors expressed on mast cells can, however, modify Fc ϵ RI-mediated mast cell activation (Hundley et al. 2004, Kuehn and Gilfillan 2007, Qiao et al. 2006). The mediators released in this manner are both diverse in nature and function. They can however be categorized into three general groups based on their mode of generation and/or release.

The first group of mediators contains those that are pre-synthesized and stored in mast cells granules (Blank and Rivera 2006, Metcalfe et al. 1997). The major such mediator with relevance to disease is histamine. However, mast cell granules also contain other bioactive amines such as serotonin, various proteases, carboxypeptidases, and other enzymes. Mast cell degranulation has been monitored by determining the extent of appearance of a number of these granule components in mast cell supernatants relative to the amount remaining in the tissues. The most widely used protocols are those designed to measure the release of histamine, the release of serotonin or the release of β -hexosaminidase. Of these, the simplest

and cheapest, and the one most amenable to the 96 well format is the β -hexosaminidase assay outlined in this unit.

The second group of mediators is the phospholipid metabolites which are generated as a consequence of the activation of phospholipase (PL) A_2 upon mast cell activation. Two subgroups of mediators are generated in this manner: platelet activating factor, and the eicosanoids; PGD $_2$, LTC $_4$, and LTB $_4$ (Boyce 2007). Eicosanoids are generated from arachidonic acid which is liberated primarily from arachidonyl-containing phosphatidylcholine by PLA $_2$. Thus, early studies looking at the generation and release of eicosanoids from mast cells, adopted a protocol whereby the cells were incubated with radiolabelled arachidonic acid, post-chasing, then determining the extent of label released following mast cell activation ((Stenson 2001) see Unit 7.33). This technique has both advantages and disadvantages. The advantages include a detection of the overall range of eicosanoids released from activated mast cells. The disadvantages include the requirement to use radioactivity or required knowledge of the use of equipment such as high-performance liquid chromatography (HPLC). More recent methods have employed the use of enzyme immunoassay (ELISA) kits and radio immunoassay (RIA) specific for each eicosanoid. Both RIA and ELISA have high sensitivity, simplicity, and specificity. However, ELISA and RIA are designed to detect only one eicosanoid, therefore a comprehensive investigation of eicosanoid generation can be expensive.

The final group of mediators released from activated mast cells are the cytokines and chemokines which are generated as a consequence of enhanced gene expression (Gilfillan and Tkaczuk 2006, Hundley et al. 2004, Metcalfe et al. 1997). The generation and/or release of these products has been assayed by multiple different approaches, both at the mRNA level and at the protein level. It should however be remembered that relative mRNA levels are not necessarily reflective of the amount of cytokine protein released from the cells. Messenger RNA levels for multiple cytokine can be simultaneously assayed either by using commercially available RNase protection assays or by multiplex gene chips. Messenger RNA levels for individual cytokines can also be measured by RT-PCR or by semi-quantitative real time PCR following design of the appropriate primers. Release of cytokines from activated mast cells is readily measured by ELISA in a similar manner to that for LTC $_4$ and PGD $_2$. However, the time of stimulation is appreciably longer (4–8 h compared to less than 30 min) than that required for degranulation or eicosanoid generation.

Troubleshooting

If there is no color change or very small color in the β -hexosaminidase release assay, the viability of the cells may be compromised. If BMMCs or HuMCs cultures are older than 7 weeks or 10 weeks, respectively, β -hexosaminidase release will begin to decrease when compared to younger cell cultures. Therefore, the cells should be used when they are 4–6 weeks (for BMMCs) or 7–10 weeks (for HuMCs). The background of β -hexosaminidase release should be less than 5 %. If the background (without stimulation) is higher than 5 %, this may be due to cells being removed with the supernatants following the centrifugation stage. Alternatively, a high background may reflect the presence of IgE aggregates, during the sensitization stage. To minimize this problem, avoid multiple freeze-thaw cycles and spin the IgE at $14,000 \times g$ at 4 °C for 60 min then collect the supernatant and store at 4 °C. Normally the IgE is stable for many months at this temperature.

Eicosanoids are lipid mediated products, thus all samples must be free of organic solvents prior to assay. If the water or buffers are contaminated with organic solvents, one may not see any color change. In this case, the source of ultra-pure water should be changed or the solution should be filtered through an organic scavenger. If one see color change in the tests samples but not the standard curve, the standard may be degraded. Eicosanoids are

chemically instable so it is easy to rapidly degrade. In this case, prepare new standard and test again.

If there is no color change in cytokine measurement, it may be due to degradation of the samples. Repeated freezing and thawing can degrade cytokines. Thus, the samples and standard must be aliquoted before freezing (-80°C). If your sample's OD is outside of standard curve, you need to increase cell numbers or dilute your sample properly.

Anticipated results

For each assay, the appropriate number of cells, incubation times, and antigen concentrations are different. The anticipated amount of mediators released from mouse BMMCs and HuMCs are given in the tables below.

Assay for BMMCs	Cells per well/100 μl	Incubation time	Antigen	Anticipated release
β -hexosaminidase	$3-5 \times 10^4$	30 min	10 ng/ml	20–50%
LTC_4	100	30 min	10 ng/ml	700–1200 pg/ml
PGD_2	2×10^3	30 min	10 ng/ml	90–200 pg/ml
Cytokine	1×10^5	6 h	10 ng/ml	IL-6: 300–600 pg/ml TNF α : 100–200 pg/ml
Chemokine	1×10^5	6 h	10 ng/ml	MCP-1: 200–300 pg/ml MIP1- α : 400–800 pg/ml

When SCF is added concurrently with antigen, degranulation, cytokine, and chemokine increases 2–5 fold compared to that produced by antigen alone.

Assay for HuMCs	Cells per well/100 μl	Incubation time	Antigen	Anticipated release
β -hexosaminidase	$0.5-1 \times 10^4$	30 min	10 ng/ml	20–60%
LTC_4	200	30 min	10 ng/ml	100–130 pg/ml
PGD_2	200	30 min	10 ng/ml	120–250 pg/ml
Cytokine	1×10^5	6 h	100 ng/ml (option: SCF 100 ng/ml)	IL-8: 200–600 pg/ml GM-CSF: 50–100 pg/ml

Time consideration

Sensitization of cells with IgE requires a minimum of 3–4 hours, maximum overnight. After sensitization, the subsequent washing steps, stimulation of cells with antigen, recovery of supernatants and total cell lysates require 3 hours for all degranulation studies. For eicosanoid generation, ELISA requires 2 days (2 hours for sample preparation, 18 hours for plate incubation, and 2 hours for development of the plate). For cytokine measurement, sample preparation requires 4–8 hours. After collection of the sample, the ELISA typically takes 4–6 hours.

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Literature cited

- Blank U, Rivera J. Assays for regulated exocytosis of mast cell granules. *Curr Protoc Cell Biol.* 2006; Chapter 15(Unit 15–11)
- Boyce JA. Mast cells and eicosanoid mediators: a system of reciprocal paracrine and autocrine regulation. *Immunol Rev.* 2007; 217:168–85. [PubMed: 17498059]
- Brown JM, Wilson TM, Metcalfe DD. The mast cell and allergic diseases: role in pathogenesis and implications for therapy. *Clin Exp Allergy.* 2008; 38:4–18. [PubMed: 18031566]
- Gilfillan AM, Tkaczyk C. Integrated signalling pathways for mast-cell activation. *Nat Rev Immunol.* 2006; 6:218–30. [PubMed: 16470226]
- Hundley TR, Gilfillan AM, Tkaczyk C, Andrade MV, Metcalfe DD, Beaven MA. Kit and FcεpsilonRI mediate unique and convergent signals for release of inflammatory mediators from human mast cells. *Blood.* 2004; 104:2410–7. [PubMed: 15217825]
- Kuehn HS, Gilfillan AM. G protein-coupled receptors and the modification of FcεpsilonRI-mediated mast cell activation. *Immunol Lett.* 2007; 113:59–69. [PubMed: 17919738]
- Marshall JS. Mast-cell responses to pathogens. *Nat Rev Immunol.* 2004; 4:787–99. [PubMed: 15459670]
- Metcalfe DD, Baram D, Mekori YA. Mast cells. *Physiol Rev.* 1997; 77:1033–79. [PubMed: 9354811]
- Qiao H, Andrade MV, Lisboa FA, Morgan K, Beaven MA. FcεR1 and toll-like receptors mediate synergistic signals to markedly augment production of inflammatory cytokines in murine mast cells. *Blood.* 2006; 107:610–8. [PubMed: 16174756]
- Stenson WF. Measurement of prostaglandins and other eicosanoids. *Curr Protoc Immunol.* 2001; Chapter 7(Unit 7–33)