Current Utilization Trend of Mast Cell Lines in In Vitro Allergy Research: A Systematic Review

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Abstract

Today in the modern world, allergic diseases, also described as atopic allergies, are classified as a type of multifactorial disorder due to the complex interplay between genetics, environment, and socioeconomic factors that influence the disease's manifestation, severity, and one's predisposition to allergic diseases. It is undeniable that many reported studies have pointed out that the mast cell is one of the main key players involved in triggering an allergic reaction. In order to improve our current understanding of the molecular and cellular mechanisms underlying allergy, various mast cell lines have been employed in vitro to study the pathogenesis of allergic diseases for the past decades. However, there is no consensus on many fundamental aspects associated with their use, such as the effects of culture media composition and the type of inducer used for cell degranulation. As the standardization of research protocols and disease models is crucial, we hereby present the outcome of a systematic review of scientific articles that used three major in vitro mast cell lines (HMC-1, LAD2, and RBL-2H3) to study allergy. This systematic review described the cell source, culture conditions, inducers used for degranulation, and mediators released for examination. We hope that the present systematic review may help to standardize the use of in vitro mast cell lines in allergy research and serve as a user's guide to understand the fundamental aspects of allergy as well to develop an effective allergy therapy in the future for the betterment of human good health and wellbeing.

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Running Head: Current Trend on Cellular Mast Cell Research

Abstract

Today in the modern world, allergic diseases, also described as atopic allergies, are classified as a type of multifactorial disorder due to the complex interplay between genetics, environment, and socioeconomic factors that influence the disease's manifestation, severity, and one's predisposition to allergic diseases. It is undeniable that many reported studies have pointed out that the mast cell is one of the main key players involved in triggering an allergic reaction. In order to improve our current understanding of the molecular and cellular mechanisms underlying allergy, various mast cell lines have been employed *in vitro* to study the pathogenesis of allergic diseases for the past decades. However, there is no consensus on many fundamental aspects associated with their use, such as the effects of culture media composition and the type of inducer used for cell degranulation. As the standardization of research protocols and disease models is crucial, we hereby present the outcome of a systematic review of scientific articles that used three major *in vitro* mast cell lines (HMC-1, LAD2, and RBL-2H3) to study allergy. This systematic review described the cell source, culture conditions, inducers used for degranulation, and mediators released for examination. We hope that the present systematic review may help to standardize the use of *in vitro* mast cell lines in allergy research and serve as a user's guide to understand the fundamental aspects of allergy as well to develop an effective allergy therapy in the future for the betterment of human good health and wellbeing.

Keywords: Allergy, Mast cells, *In vitro* , HMC-1, LAD2, RBL-2H3

1.0 Introduction

Humans have battled with allergies for decades. The concept of allergies was first introduced in the early twentieth century by an Austrian scientist named von Pirquet, describing that an allergy occurs when exogenous substances (allergens) induce a change in reactivity in an individual's immune system, leading to hypersensitive reactions[1]. Typical allergens are found in a wide range of environmental substances varying in their nature and source, including food allergens, aeroallergens like pollen, mites, and dust, as well as chemical allergens like dyes, creams and skincare products (2). Common allergic diseases include atopic eczema (dermatitis), rhinitis, allergic asthma, drug, and food allergies. Allergic diseases are considered a worldwide severe health issue, and their prevalence comprises a substantial percentage of the population. Rhinitis and food allergies affect 10-30% and 8% of the population worldwide, respectively, while skin allergies such as eczema have a lifetime prevalence of 20% worldwide [2]. Allergy symptoms range from mild, such as itchiness, hives, watery eyes, and a runny nose, to life-threatening outcome, depending on the hyperreactivity of the immune system. The lethal and exaggerated allergic reaction known as anaphylaxis is the primary cause of death in allergic patients [3].

Currently, the most well-known curative treatment for IgE-mediated allergies is allergen-specific immunotherapy (AIT). This form of therapy involves subcutaneous administration of gradually increasing quantities of a patient's corresponding allergen until an ideal dose capable of stimulating immune tolerance toward the allergen is achieved [4]. Immunologic improvements in patients subjected to AIT are associated with the production of T regulatory cells that induce the anti-inflammatory cytokine IL)-10, which causes an early decrease in mast cells and basophil activation and the subsequent reduction of inflammatory mediators such as histamine[5]. Despite the efficacy of AIT, the development of immune tolerance in patients is still an

evolving area. Other short-lived first-line treatments widely used consist of inhalation of corticosteroids, β-adrenergic agonists, and leukotriene modifiers in allergic asthma, or the avoidance of the food allergen and treatment with antihistamines for mild symptoms of food allergies are available[6,7]. However, these forms of treatment merely alleviate allergy symptoms rather than target the underlying pathology of the disorder.

The use of *in vitro* mast cell models may be able to answer and resolve some of the issues faced with current treatment. Mast cells have been considered the main effector cells in allergic reactions, and as a result, they have become attractive candidates in the study of allergenicity and sensitization mechanisms. Mast cells originate from multipotent hematopoietic stem cells that are mainly distributed in blood vessels located at the host-environment interface, such as the skin, airways, and gastrointestinal tract. Their localization in the body makes them one of the first immune cells to interact with incoming allergens [3]. As described previously, mast cells play a central role during an allergic reaction. As mast cells are replete with the high-affinity IgE receptor FcεRI, binding of allergen-specific IgE stimulates mast cell degranulation releases prestored pro-inflammatory mediators such as histamine, serotonin and proteases as well as *de novo* synthesis of inflammatory mediators such as leukotriene and prostaglandins [8]. This surge in the excessive release of such mediators rapidly triggers anaphylactic shocks. As mast cells differentiate in the peripheral tissues from progenitor cells in the bone marrow, $CD34^+$ myeloid progenitor cells, derived from buffy coats, cord blood, or bone marrow, have been used as the primary source for the generation of mast cells*in vitro* [9]. However, *in vitro* research using human mast cells pose several challenges such as having low proliferative activity and the differentiation steps occurring physiologically in tissues are time-consuming, difficult and expensive to recapitulate *in vitro* [10]. As such, several commercial human mast cell lines have been generated such as the HMC-1 (human mast cell line 1), LAD2 (Laboratory of allergic diseases 2) and LUVA (Laboratory of University of Virginia) as well as rodent mast cell lines such as the RBL-2H3 (rat basophilic leukemia-2H3) cell line which are routinely used as *in vitro* allergy models depending on their specific advantages and limitations [11]. Although no model has been able to fully replicate human mast cell phenotypes, given the right culturing conditions and experimental setup, each model may possess some benefit over the selection of others.

To our knowledge, there is relatively limited data on large-scale tabulated data regarding the usage of mast cell line models. Hence, in this paper, three common mast cell line models (HMC-1, LAD2, and RBL-2H3) used in allergy-related studies are systematically reviewed with respect to their culturing conditions, types of inducers used, and inducing conditions. We also draw comparative tabulations and reasonings on the type of mast cell line used in respect of the type of laboratory conditions and experimental purposes. The review seeks to provide researchers with details on the characteristics and mechanisms of each mast cell model to aid in the proper selection of models for future studies.

2.0 Methods

2.1 Search Strategy

Relevant articles were identified from two different databases (Web of Science and ScienceDirect) using keywords: allergic inflammation AND mast cell activation. All reported research studies that use *in vitro* mast cells were included in this systematic review. Filter was applied to include research articles in English that were published from 2018-2023. This study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) 2020 guidelines. The last search for relevant articles in all databases was performed on 15 December 2023.

2.2 Eligibility Criteria

For this systematic review, the inclusion criteria are (1) *in vitro* mast cell studies, (2) secondary cell line culture studies, and (3) allergy-related studies. Only published research articles from 2018-2023 were included. On the other hand, the exclusion criteria are (1) animal work that do not involve any *in vitro* mast cell studies, (2) only involve primary cell line culture studies, and (3) full-text not accessible.

2.3 Study Appraisal and Selection

All the articles obtained from the databases using the specific keywords were organized according to their titles, and duplicates were identified by the same title, authors, and year of publication. The redundant studies were removed, and the remaining articles were screened using the pre-defined eligibility criteria. Firstly, the title and abstract of each article were assessed by two reviewers independently. Those that matched the eligibility criteria were then subjected to full-text screening to further determine their relevance. Disagreements between the two reviewers throughout the screening process were resolved by consensus, and the reasons for excluding the articles were recorded.

3.0 HMC-1 cell line

The human mast cell 1 line (HMC-1) is a well-known model used in allergic and inflammatory disease studies. It was established from a mast cell leukemia patient, entailing a dedifferentiated, spindle-shaped hypogranular appearance [9,12]. While it is considered an immature mast cell, the HMC-1 cell line shares a phenotype akin to human mast cells (HuMC). The expression of mast cell-associated markers (i.e., histamine, heparin, tryptase, and c-kit receptor) and a similar cell surface antigen profile render the HMC-1 cell line a strong candidate in allergy research[13,14]. HMC-1 can be further subdivided into HMC-1.1 (HMC^{560}) and HMC-1.2 ($HMC^{560,\ 816}$), depending on the location of the mutation in the c-kit receptor. HMC-1.1 possesses a substitution of glycine-560 to valine (V560G), while HMC-1.2 contains the V560G mutation and another substitution of valine-816 to aspartate (D816V) [15,16]. These mutations in the c-kit receptor cause dysregulation in c-kit receptor expression, thus resulting in the survival of HMC-1 cell lines independent of stem cell factor (SCF) which is essential for mast cell proliferation, chemotaxis, activation, differentiation, and survival [17,18]. This allows the usage of HMC-1 cells to be highly favorable in an *in vitro* mast cell research as they have a higher proliferative rate than other mast cell lines. The doubling time of the HMC-1 cell line (1-3 days) is described to be 10-fold faster than the LAD2 cell line (10-14 days)[9,19]. In addition, when compared between the two variant sublines, HMC-1.2 exhibits a higher proliferative rate than HMC-1.1 due to the presence of D816V mutation [20]. This mutation leads to constitutive tyrosine kinase activation of the Kit receptor that causes a higher proliferation rate as compared to the HMC-1.1 which does not have the same mutation point[21].

Despite sharing a phenotype akin to HuMCs, the HMC-1 cell line contains dissimilarities when compared to mature HuMCs. HMC-1 cell line has a low expression of mature HuMCs markers (i.e., tryptase and chymase), with the exception of c-kit and histamine, representing immature malignantly transformed mast cells [22]. The binding of IgE to the high-affinity IgE receptor FcεRI is crucial for the activation and degranulation of mast cells [23]. As such, the lack in surface expression of FcεRI in the HMC-1 cell line requires the usage of a physiological stimulus, such as phorbol myristate acetate (PMA), calcium ionophore (CI), and compound 48/80 (C48/80)[24]. Additionally, Mas-Related G-protein coupled Receptor X2 (MRGPRX2) is known to induce mast cell degranulation, contributing to pseudo-allergic reactions caused by small molecule drugs. The HMC-1 cell line, however, expresses lower levels of MRGPRX2 and poor degranulation activity than the LAD2 cell line and HuMCs. It is noted that latrunculin-B can be used to prompt MRGPRX2-mediated degranulation [9]. Overall, the shortcomings of the HMC-1 cell line may potentially limit the insights available to mast cell activation studies. Yet, the high proliferative rate and stable phenotype render them a feasible model for extensive *in vitro*studies.

3.1 Cell source and culture conditions of HMC-1 cell line

The HMC-1 cell line is often sourced from cell banks (20 of 50), with the most provided by Dr. Joseph H. Butterfield from Mayo Clinic (6 of 50) followed by American Type Culture Collection (ATCC) (5 of 50), Sigma-Aldrich (2 of 50), and Korean Cell Line Bank (KCLB) (2 of 50). Other cell banks include the Cellcook Biotechnology (CB), National Platform of experimental cell resources (NPECC), National Centre for Cell Science (NCCS), Wu-Han University Cell Collection Center (WUCCC), and Chinese Academy of Sciences (CAS). In addition, HMC-1 cell line from 11 out of 50 publications were gifted by other institutions, with the most from Eiichi Morri Osaka University (4 of 50). Following that, Prof. Jae-Young Um from KyungHee University and Prof. Jong-Sik Jin from Jeonbuk University contributed 2 out of 50 publications each. Other institutes include Hoseo Univeristy (Prof. Hyun-Ja Jeong), Second Military Medical University (ZhiLiang

Yu), and Sangji University. However, the authors did not report the original source of their gifted HMC-1 cell line. Finally, 19 of the shortlisted publications did not specify the cell origin. The sources of HMC-1 are summarized in Figure 2.

The composition of the growth medium used for HMC-1 cell line propagation varies among publications. Sigma-Aldrich and MERCK recommend HMC-1 cell line to be maintained in IMDM supplemented with 10% Fetal Bovine Serum (FBS), 1.2mM α-thioglycerol, and 1× penicillin/streptomycin in a 37°C humidified environment with 5% CO_2. In actual protocols employed in the publications, most of the articles maintain HMC-1 cell line in IMDM (43 of 50), followed by RPMI-1640 (5 of 50), DMEM (1 of 50) and IMEM (1 of 50). In addition, the media was supplemented with 10% FBS (45 of 50), penicillin/streptomycin (44 of 50), 2mM L-glutamine (4 of 50), monothioglycerol (3 of 50), α-thioglycerol (2 of 50), 10% fetal calf serum (FCS) (2 of 50), 2-mercaptoethanol (1 of 50), amphotericin B (1 of 50), and sodium bicarbonate (1 of 50). Table 1 summarizes the culture medium and conditions to grow HMC-1 cells.

The choice of medium and composition are crucial to provide an optimal environment for cell growth and survival. IMDM, a highly enriched synthetic medium, is often recommended for rapidly proliferating and high-density cell lines. While no studies have reported the correlation between medium composition and the growth of HMC-1 cells, evidence shows that DMEM and RPMI-1640 media can affect the growth and differentiation of several cell lines [25,26]. The medium's excess or lack of calcium ion (Ca^{2+}) and inorganic phosphate (Pi) may attenuate the differentiation of several cell lines. The concentration of 1.8 mM and 0.09 mM of Ca^{2+} and Pi, respectively, are optimum for cell proliferation [27]. L-glutamine is supplemented to the medium to serve as an energy source for rapidly dividing cell lines[28]. The degradation of L-glutamine to ammonia may be toxic to the cells, where 2 to 3 mM is sufficient to reduce cell growth. Yet, such occurrence is dependent on the cell line [29]. In addition, there are contradictory reports on supplementing media with L-glutamine on cytokine release. Coëffier et al. (2001) demonstrated the reduction in pro-inflammatory cytokines (IL-6 and IL-8) from human intestinal mucosa by glutamine via a post-transcriptional pathway[30]. Glutamine has also decreased the expression of leukotriene C_4, monocyte chemoattractant protein (MCP), macrophage inflammatory protein (MIP)-1β, tumor necrosis factor alpha (TNF-α), interleukin (IL)-15, and IL-18 in human intestinal mast cells, and lobectomy patients [31,32]. In contrast, several publications observed an increase in Th1 cytokines (IL-2 and IFN-γ) in PMACI-treated intestinal intraepithelial lymphocytes by glutamine[33]. Similarly, IL-1 and IL-10 were upregulated in glutamine-treated lobectomy patients [32]. Other supplements, such as sodium pyruvate in IMDM, have been shown to impair cytokine production by inhibiting inflammatory signalling pathways[34].

3.2 Inducers and mediators release for HMC-1 cells

As the HMC-1 cell line lacks the expression of FcεRI, an external stimulus is required to induce mast cell degranulation. The combination of PMA and CI is widely used (25 of 50 and 28 of 50 respectively). Other inducers include OVA, IL-33, TSLP, protein kinase activator C, RANKL, LPS, histamine, and more. However, three of the 50 publications did not specify the type of inducers used. The induction time implemented by most studies falls within 5 to 8 hours (h) (23 of 50), followed by an hour and below (20 of 50), within 13 to 24 h (16 of 50), 2 to 4 h (13 of 50) and 9 to 12 h (2 of 50). However, four publications did not indicate the induction time. The degranulation of mast cells can be characterized by the concentration of cytokines secreted. Many of the publications studied the release of TNF-α (29 of 50), IL-6 (27 of 50), IL-1β (20 of 50), histamine (17 of 50), TSLP (14 of 50), and IL-8 (12 of 50). Table 2 summarizes our analysis of the type of inducers, induction time, and type of mediators often studied by researchers using HMC-1 cells.

Mast cell activation depends on the cross-linking of IgE antibodies on the FcεRI and the subsequent signal transduction cascade, including intracellular Ca^{2+} mobilization, influx, and protein kinase C (PKC) activation. As such, the combination of PMACI is a strong candidate as an inducer owing to its ability to enhance Ca^{2+} influx and activation of MAP kinases [35,36]. PMA stimulates PKC activity, while CI raises the intracellular level of Ca^{2+} [37,38]. Many clinical studies have shown the upregulation of cytokines released (Th1-related cytokines) upon PMACI stimulation, namely the IL-2, IL-6, IL-17, IFN-γ, and TNF-α. However, these studies did not affect the expression of IL-4 and IL-10 [39–41]. Another point to note in future research is that the

expressions of IL-10, IL-17, IL-22, IFN-γ, and TNF-α are higher in IMDM than RPMI-1640 upon PMACI stimulation due to the concentration of Ca^{2+}. According to Zimmermann and his colleagues (2015), IMDM consists of 1.49 mM of Ca^{2+}, while RPMI-1640 contains 0.42mM of Ca^{2+}. Therefore, by increasing the Ca^{2+} concentration in RPMI-1640 to 1.5 mM, the expression of cytokines is comparable to IMDM. Vice versa, the reduction of Ca^{2+} concentration in IMDM resulted in a lowered cytokine expression. Thus, implying that 1.5 mM of Ca^{2+} is optimal for maximal ionomycin stimulation in cells [42].

When compared between PMACI, lectin phytohaemagglutinin (PHA), LPS, Con-A, and pokeweed mitogen, PMACI expresses the most potent cytokine production in a short period without significant damage to the cells[43]. On the other hand, C48/80 acts as a "selective" mast cell activator by stimulating the trimeric G-proteins and activating phospholipase C and D pathways [44]. C48/80 activation can bypass Ca^{2+} and PKC signal transduction, thus beneficial when PMACI cannot be used[35].

4.0 LAD2 cell line

Prior to the discovery of LAD cells, HMC-1 cells were the only cell culture available to researchers that resembled human mast cells. However, HMC cells' usefulness is limited by two deficiencies – they are growth factor independent and they degranulate inconsistently to IgE-dependent signals possibly due to the variable expression of the FcεRI α-subunit [45]. Laboratory of Allergic Diseases 2 (LAD2) human mast cells were first discovered through a routine study of cells from bone marrow aspirates of a mast cell sarcoma/leukemia patient[45]. During this routine study, researchers discovered cultures of mast cells with functional FcεRI and FcγRI receptors that continue to proliferate in a stem cell factor-containing serum-free media. These cells resembled $CD34^{+}$-derived human mast cells and responded to human recombinant c-kit receptor ligand stem cell factor (rhSCF) while sharing similar characteristics with LAD1 cells. Morphologically, LAD2 cells stained with acid toluidine blue and tryptase are oval or round nucleated cells with metachromatically staining granules and they measured between 8 to 15 μm diameter. Under the electron microscope, they appeared as cells with rough surfaces and cytoplasmic projection [45]. LAD cells highly resemble mast cells as they expressed surface FcεRI, cluster of differentiation (CD) 4, 9, 13, 22, 45, 64, 71, 103, 117, 132, C-C chemokine receptor type 5 (CCR5) and C-X-C chemokine receptor type 4 (CXCR4) and CD14, 31 and 32 on a lesser degree. They can release histamine and β-hexosaminidase upon FcεRI aggregation. To date, LAD2 cells have been used to study mast cell proliferation, receptor expression, mediator release/inhibition during mast cell degranulation, and also signaling[19,46]. In addition, these cells are commonly used in studies involving MRGPRX2. The MRGPRX2 is expressed by mast cells and degranulates upon binding by different ligands; and is involved in pseudo-allergic reactions, chronic spontaneous urticaria, atopic dermatitis and allergic asthma [9].

LAD2 cells degranulate well when stimulated but they lack the ability to generate cytokines as personally experienced by Rådinger et al. (2010). Apart from that, Rådinger et al. (2010) also noted the relatively slow growth rate of LAD2 cells – doubling rate of approximately 2 weeks. Although LAD2 cells require longer time to proliferate compared to some tumorigenic cells which take 3 to 5 days; Kirshenbaum et al. (2003) believed that this longer duration allowed the cells to exhibit a more mature phenotype. Other potential drawbacks of LAD2 cells are excessive clumping when the cells were grown for a prolonged duration and the slower growth may hampered the cells' responsiveness to biotinylated IgE/streptavidin crosslinking and thus reducing activation and degranulation. However, this could be easily overcome by maintaining the cell concentrations between 0.25-0.5 $\times$ 10^6 cells/mL to reduce cells clumping and performing hemidepletions every 3-4 days as suggested by Rådinger et al. (2010) and to freeze down cells frequently, then thaw and expand a new stock culture yearly [45].

4.1 Cell source and culture conditions of LAD2 cell line

LAD2 cells are often sourced from the Laboratory of Allergic Diseases, National Institutes of Health (NIH) in Bethesda, United States of America (USA) where the cells were first discovered and successfully cultured. Of the 75 papers analysed, 55 of the research groups obtained their LAD2 cells from NIH, specifically the laboratory of Drs Arnold Kirshenbaum and Dean Metcalfe [47]. Some were obtained from Otwo Biotech

company (1 of 75), Dr Yangyang Yu of Shenzhen University (1 of 75), Dr Michael of Colombia University and Professor Renshan Sun of Third Military Medical University (1 of 75). However, the authors did not report the original source of their gifted LAD2 cell line. Finally, 17 research groups did not specify the origins of their LAD2 cells. Figure 3 summarizes the most common sources to obtain LAD2 cells.

StemPro-34 is the culture medium used to culture LAD2 cells as observed in most of the 75 papers analysed (74 of 75). There is only one publication that reported the use of IMDM with supplementation of 10% FCS to culture the LAD2 cells. StemPro-34 is a specifically formulated serum-free medium used to support the growth of human hematopoietic cells. Supplements are often added in the StemPro-34 culture medium for LAD2 cells. The medium is usually supplemented with StemPro-34 nutrient supplement which include pre-mixed penicillin/streptomycin, L-glutamine, and human stem cell factor. Apart from that, Zou et al. (2022) added 50 ng/mL of IL-6 into their StemPro-34 medium. Cytokines and growth factors are added into the medium to further support the growth of the progenitor cells. The use of StemPro-34 is in accordance with the medium used when LAD2 cells were first cultured in the laboratory of Drs Kirshenbaum, Akin and Metcalfe. Similarly, the medium was supplemented with 2 mM L-glutamine, 100 IU/mL penicillin, 50 µg/mL streptomycin, 100 ng/mL rhSCF. The addition of 100 ng/mL of rhIL-6 and 30 ng/mL rhIL-3 for the first week were optional [45]. Kirshenbaum et al. (2003) also noted that even though LAD2 cells could survive in medium without SCF, the numbers doubled in approximately 3 weeks with 100 ng/mL SCF, and the addition of rhIL-3, rhIL-5 or rhIL-6 did not influence the cell numbers. SCF is needed for the growth of LAD2 cells as they do not possess the *c-kit* activating mutation at codon 816. LAD2 cells were grown in a humidified incubator at 37°C with 5% CO_2. Table 3 summarized the culture medium used to grow LAD2 cells. As suggested by Rådinger et al. (2010), hemidepletions of the medium were performed by most researchers when growing the LAD2 cells at the frequency of once a week. The cell density was generally maintained between 1×10^5 to 2×10^6 cells/mL.

4.2 Inducers and mediators release for LAD2 cells

As LAD2 cells express the high affinity IgE receptor, they can be sensitized with antibody to induce degranulation. Among the 75 shortlisted articles, only 24 of them reported that they sensitized LAD2 cells with the IgE antibody. Other studies directly activate the LAD2 cells to degranulate using their chosen inducer(s). Several inducers have been used in studies involving LAD2 cells. This includes the common C48/80 which was being used by 36 of the studies. C48/80 is a known mast cell degranulator that has been used in countless allergy-related studies [48]. Substance P is also commonly used as an inducer of LAD2 cells (14 of 75). Substance P is an undecapeptide found in the human body and it is secreted from the terminals of specific sensory nerves [49] and it is a known ligand of MRGPRX2[50]. A major product of the adrenomedullin precursor, PAMP(9-20) [49] was used by three research groups to induce LAD2 cells in this analysis. Other inducers that were being used to activate LAD2 cells include Tween 20, LL-37, (R)-ZINC-3573, CST-14, Thapsigargin and complement C3a as shown in Table 4. Apart from these common inducers, several studies have analyzed the degranulation effects of antidepressants (clomipramine, paroxetine and desipramine), contrast media (gadopentetate meglumine, iodinated contrast media and iopamidol), p-Phenylenediamine (PPD), P17, morphine derivatives (thebaine and pethidine) and fluoroquinolones and many others. PPD is a component found in hair dyes implicated to induce immediate allergy, acute dermatitis and contact dermatitis[51]. On the other hand, P17 is a short host defense peptide from the ant *Tetramonium bicarinatum* venom[52]. LAD2 cells are commonly used to study MRGPRX2 related pseudo-allergy reactions and thus they were used to identify several novel mast cells compounds [53]. Besides that, the effects following exposure to influenza A virus were also studied using LAD2 cells [54]. As LAD2 cells are mast cells, some researchers chose to pre-sensitize the cells first before inducing them with the corresponding inducers. These studies used DNP-IgE as sensitizer (18 of 75) and then followed by either DNP-HSA, DNP-BSA, DNP-streptomycin, or streptavidin as inducers in their studies. Other combinations such as biotinylated IgE with streptavidin and human myeloma IgE with anti-IgE were also used by several researchers.

From the articles analyzed, histamine (36 of 75) and β-hexosaminidase (62 of 75) are the two common mediators that were often evaluated. Both mediators are pro-inflammatory mediators that play key roles as

degranulation biomarkers [55]. Other mediators include cytokines and chemokines. Thirty out of 75 articles evaluated the levels of MCP-1, while 28 analyzed TNF-α. Several ILs were analyzed too, and these were IL-8 (27 of 75); IL-6 (5 of 75) and others. Several other mediators evaluated include 5-hydroxytryptamine (5-HT), tryptase, MIP-1α, MIP2, prostaglandin 2 (PGD2), leukotriene B4 (LTB4), granulocyte-macrophage colony-stimulating factor (GM-CSF) and many others. The induction time differs based on the type of mediators studied. From our analysis, generally, LAD2 cells were induced between 0-1 h when β-hexosaminidase and histamine were to be evaluated. The duration of 30 minutes (mins) was the most common induction period while some groups induced the cells for 40 mins, with the longest being 2 h[56]. The shortest duration used to stimulate LAD2 cells to produce β-hexosaminidase was 15 mins by Park et al. (2019) and histamine for 10 mins by Sun et al. (2021) [57,58]. For the evaluation of late mediators such as TNF-α, ILs, MCP-1 and others, longer induction time was needed, and it ranged from 6 to 8 h, with 6 h being the most used induction time. Two other induction times were noted which were 12 h and between 24 and 48 h. In the study involving influenza A virus, the cytokines and chemokines were analyzed at days 1, 2 and 4 (maximum) post infection [54]. In another study using 12 h induction time, the researchers were evaluating the effects of substance P and PAMP(9-20) on LAD2 cells and analyzed the levels of TNF-α, MCP-1, IL-8 and IL-31 [49]. On the induction time of 24-48 h, the study studied the effects of P17 on LAD2 cells, analyzing MCP-1 and MIP-1α release [52]. Table 4 summarizes our analysis on the type of inducers, induction time and type of mediators often used and studied by researchers using LAD2 cells.

5.0 RBL-2H3 cell line

In mast cell research, the most common animal cell line used is the RBL-2H3 cell line[59]. The RBL cells were generated from rats that were injected with the chemical carcinogen β-chlorethylamine to induce basophilic leukemia [60]. The RBL cells were adapted to suspension cell culture (named RBL-1) and can specifically bind IgE to their surface membrane [61,62]. Nevertheless, neither an IgE/antigen trigger nor chemical stimulation by a Ca^{2+}ionophore were able to cause RBL-1 to release histamine[63]. A responsive subline known as RBL-2H3 that degranulated in response to an IgE trigger was successfully isolated from subsequent cloning of RBL cells in 1981 [64]. These cells were a great model for understanding the FcεRI signaling cascades as they could be cultured in huge numbers to examine the characteristics and binding properties of IgE which will lead to the signaling pathways involved in degranulation [65].

RBL-2H3 cells were widely employed as a mast cells model shortly after they were formed. Unquestionably, RBL-2H3 cells have the advantage of being a cell line that is simple to cultivate due to its short doubling time (18-24 h), enabling researchers to obtain a high number of homogenous cells easily. However, their suitability and credibility were eventually questioned [66,67]. In the early years of RBL cells establishment, a variant with impaired cromoglycate binding had been identified in a population of RBL-2H3 which suggest that the cell line may not be fully homogenous [68]. Although research reports frequently referred to RBL cells as mast cell line, it was in fact derived from basophils. Additionally, the cell line was lack of consistency as to the physiology of basophils or mast cells as well as findings between other research groups utilizing the same cell line[66]. Mast cells and basophils are two different but functionally related cell types that are essential in type I hypersensitivity. Granulocyte basophils circulate, whereas mature mast cells are only found in tissues at the interfaces with external environment, such as the lungs, skin, and mucosal surfaces[69]. Despite their similarities, it was suggested that the two cell types descended from distinct lineages[70,71]. Nonetheless, numerous arguments suggested that they may share a common origin [72,73].

Nevertheless, RBL-2H3 cells have been successfully used in investigations on IgE binding to FceRI receptors and subsequent downstream processes [66,74]. The expression of rat mast cell protease II (RMCP-II) [75] and a similar expression of the c-kit receptor tyrosine kinase [76] in RBL-2H3 cells comparative to human HMC-1 and murine P-815 mast cells were one of the few aspects of mast cell physiology that supports the notion that RBL-2H3 cells can model mast cells. Moreover, numerous studies have found similarities between the exocytosis mechanisms of bone marrow mast cells and RBL cells involving SNARE proteins, designating RBL cells as a suitable model for research on MC exocytosis [59].

5.1 Cell source and culture conditions of RBL-2H3 cell line

Most of the RBL cell lines used in allergy research were the RBL-2H3 cell line (131 of 134), followed by humanized RBL cells (3 of 134). The American Type Culture Collection (ATCC) is the most reported source (46 of 131) for the RBL-2H3 cell line. Other cell bank sources of RBL-2H3 includes China Center for Type Culture Collection (CCTCC) (20 of 131), Japanese Collection of Research Bioresources Cell Bank (JCRB) (9 of 131), Korean Cell Line Bank (KCLB) (7 of 131), Procell Life Science & Technology (PLCT) (3 of 131), Bioresource Collection and Research Center (BCRC) (2 of 131), Chinese Academy of Sciences Shanghai (CAS) (2 of 131), Binsui Biotechnology (SBB) (1 of 131), Cellcook Biotechnology (CB) (1 of 131), Shanghai EK-Bioscience Biotechnology (SEB) (1 of 131), National Infrastructure of Cell Line Resource (NICLR) (1 of 131), and National Experimental Cell Resource Platform (NECRP) (1 of 131). Additionally, cells were received as gifts from scientists from other universities (4 of 131). However, the authors did not report the original source of their gifted RBL cell line. Finally, the cell origin was not specified in 33 out of 131 publications. Figure 4 summarizes the cell line sources of RBL-2H3.

ATCC and BCRC recommends RBL-2H3 to be cultured in MEM that contains Earle's Balanced Salt Solution, non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate, made complete with the supplement of 15% FBS. CCTCC and JCRB recommend using the same MEM base media with slight reduction of FBS to 10%. KCLB, however recommends DMEM with 10% FBS. For institutions other than the aforementioned above, there is no information regarding the recommended media used for the culture of RBL-2H3.

Review of published methods revealed significant variation in the culture media and supplements used to cultivate RBL-2H3 cells (Table 5). However, 11 out of 131 studies did not disclose the culture conditions used. DMEM was the most used (69 of 131), followed by MEM (44 of 131), MEM Eagle-alpha modification (2 of 131), RPMI-1640 (3 of 131), and EMEM (1 of 131). For serum, media were mostly supplemented with 10% FBS (91 of 131), followed by 15% FBS (26 of 131), 20% FBS (1 of 131) 17% FBS (1 of 131) and 5% FBS (1 of 131). Other supplements reported include penicillin/streptomycin (102 of 131), L-glutamine/GlutaMAX (16 of 131), Sodium pyruvate (14 of 131) and non-essential amino acid (NEAA) (7 of 131). Two study utilized distinctive medium formulation for the culture of RBL-2H3 cells: a concoction of 70% α-MEM, 20% RPMI 1640 and 10% FBS with 2mM L-glutamine, as well as a mix of 41.5% MEM, 41.5% IMDM and 17% FBS with 25 mM HEPES and 120 μg/mL gentamicin sulphate. There is yet to be any report on the influence of various media and supplements on RBL-2H3 cells physiology and function.

5.2 RBL-2H3 cell sensitization, induction, and degranulation

Following the crosslinking of their IgE-bound FceRI by multivalent allergens, RBL-2H3 cells, like mast cells and basophils, respond with degranulation, releasing a variety of mediators that trigger a potent immunological allergic response[77]. The cells were demonstrated to have a bell-shaped dose-response to anti-DNP IgE as observed in primary mast cells[78,79]. A range of non-immunological triggers such as the C48/80 and A23187 can also cause RBL-2H3 cells to degranulate[80]. Calcium ionophore A23187 can induce mast cell degranulation by increasing calcium entry into the cells through pore formation or as a transporting carrier [81]. RBL-2H3 cells behave similarly to basophils and mast cells when exposed to the A23187 [82,83]. A23187 at a concentration of 5 μg/mL caused RBL-2H3 cells to degranulate about 50% of available histamine, which is 1.65× more than that of IgE [67]. On the other hand, degranulation of MCs, especially connective tissue MCs can be induced by polybasic compounds like C48/80. C48/80 can interact with G_0 and G_i and their subfamilies of G-protein coupled receptors (GPCRs) to activate their downstream signaling of degranulation [84–86]. However, polybasic compounds were found to be inactive on some MC subfamilies, including RBL cells which may be due to their lack of $G_{i\text{-}3}$[87,88]. Nonetheless, depending on the culture conditions, they can develop sensitivity to C48/80. Interestingly, quercetin can cause a rise in the expression of $G_{i\text{-}3}$'s subunits, triggering RBL-2H3 response to C48/80 [88]. Another study also reported that RBL-2H3 cells change into a C48/80 active phenotype when co-cultured with 3T3 fibroblast[89]. Unfortunately, they were unable to ascertain the factors implicated with this phenomenon.

Review on the assays performed using RBL-2H3 cells (Table 6) demonstrated that most of the studies sensitize the cells with DNP-BSA and later induce the cells with DNP-HSA (91 of 131) and measured the

degranulation of β-hexosaminidase (115 of 131) and histamine (61 of 131). The induction times were mainly reported to be within 1 h (91 of 131), with most of the studies reportedly induced RBL cells for a full 1 h (29 of 131). One study used RBL-2H3 cells for cytotoxicity assay but did not investigate any mediators released nor used any inducers. β-hexosaminidase is an exoglycosidase enzyme that is associated with degranulation, and it is released together with histamine[80]. Based on our review, β-hexosaminidase is the most common marker used to measure degranulation for RBL-2H3 cells. On the other hand, histamine had a significant role in allergy and inflammatory reactions as it mediates the interactions between inflammatory cells[90]. The pre-stored histamine within the granules of RBL-2H3 cells were inconclusive as wide range of histamine content had been reported per 1×10^5 cells: from 20-45 ng[91], to 100 ng [92] and up to 700 ng[67]. MCs had been known to produce pro-inflammatory cytokines like IL-4, IL-13 and TNF-α through the induction of TLR4 and TLR2 pathways [93]. RBL-2H3 has been observed to release up to 180 pg/mL of IL-13 and 60 ng/mL of TNF-α [94]. Our review revealed that 48 of 105 publications had investigated and demonstrated cytokines production in RBL-2H3 cells. However, there are studies reported conflicting findings that RBL-2H3 cells did not respond to lipopolysaccharide induction [66,67]. Those studies also demonstrated that RBL-2H3 cells did not expressed CD14 and MyD88 that is implicated in TLR4 signaling pathway. Additionally, they also reported that RBL-2H3 cells were also unresponsive to TLR2 ligands due to the lack of TLR2 receptors [66,67].

5.3 Humanized RBL cells

As discussed above, RBL cells originated from rats. It was established that rat IgE can bind to human IgE receptor [95], however rat IgE receptor did not recognize human IgE [96]. RBL cells expressing the high affinity IgE receptor FcεRI implied that the cell line may be suitable for the detection of allergen specific IgE and allergens[59]. Stable transfection of human FcεRI α chain in RBL cells was achieved in the 90s with the receptor expressed mediated antigen-induced signaling and mediator release[97–100]. These humanized RBL cells were then utilized to investigate the ability of pure peanut allergens and other food allergens to induce degranulation [101–104]. RBL-SX38 is one of the commonly used humanized RBL cell lines that expresses α, β, and γ chains of human FcεRI [105].

The addition of a reporter gene (firefly luciferase) to humanized RBL cells paved the way for straightforward and extremely sensitive detection of cellular activity via the IgE receptor[59]. The first and most common system was a Nuclear Factor of Activated T-cells (NFAT)-firefly luciferase reporter that is linked to IgE dependent signal transduction named RS-ATL8 cells [106]. The calcium influx from stimulation of the cells activates calcineurin, a phosphatase that dephosphorylates NFAT. This resulted in the unveiling of the nuclear localization signal in the N-terminal and leads to nuclear translocation. NFAT then attaches to specific promoter regions and initiates gene transcription of the luciferase reporter gene[107]. By detecting luciferase activity with suitable substrates and a luminescence detector, activation of these cells may be detected [106]. RS-ATL8 assay is very sensitive and well suited for high throughput format. The assay had since been employed for the identification of food or other allergens, as well as determining the allergenicity of vaccines [108–110].

From our literature retrieval, we identified two articles using the humanized RBL cells, RBL-SX38 [111,112] and one article using the reporter humanized cells, RS-ALT8 [113] to investigate the release of β-hexosaminidase. RBL-SX38 was obtained from a research group in Harvard Medical School, USA while the study using RS-ALT8 did not specify the cells' origin. Both RBL-SX38 and RS-ALT8 cells were grown in MEM supplemented with 10% FBS, L-glutamine and antibiotics. One study using RBL-SX38 did not specify the culture condition. In all three studies, cells were sensitized with allergen specific human IgE and induced by their respective allergens.

6.0 Alternative options for *in vitro* mast cell studies

Apart from the mentioned cell lines, there are also other alternative options for *in vitro* mast cell studies in allergy albeit not as commonly reported. One of them is the Immortalized Human Mast Cell Line (LUVA). This cell line was first identified and characterized by Laidlaw and her collegues in 2011 [114]. The cells were grown from CD34+-enriched mononuclear cells derived from the peripheral blood of a donor with aspirin

exacerbated respiratory disease[114]. The authors mentioned that this cell line can be maintained without stem cell factor to survive and proliferate without further addition of any growth factors for approximately 2 years. In addition, LUVA cells also display high levels of normally signaling c-kit, metachromatic cytoplasmic granules, and FcεRI[114]. These cells will prove valuable for functional human mast cell studies as they can be induced to degranulate and release various allergic mediators such as β-hexosaminidase, prostaglandin D2, thromboxane A2, and MIP-1β.

Another human mast cell line that is suitable for allergy studies is the ROSA^{KIT} mast cells. This cell line was first reported in 2014 [115]. Unlike LUVA cells, these cells require stem cell factor to survive and proliferate with a doubling time of 24 h[115]. ROSA^{KIT} cell also has functional IgE receptors which allows it to be easily activated to release mediators whenever FcεRI crosslinking happens[115]. Apart from activation through IgE receptors, this human mast cell line can also be induced by means of calcium influx. A reported study showed that the ROSA^{KIT} cell induction using calcium ionophore A23187 was able to release 80% of β-hexosaminidase as compared to using IgE-FcεRI which only releases approximately 38% of the enzyme after 1 h induction[115].

Apart from that, secondary mast cell lines from mouse origin such as MC/9 and P815 cell lines are also available. The MC/9 is a cloned mast cell line derived from the fetal liver of a (B6 × A/J)F1 mouse[116]. These cells can be sensitized to specific antigens by incubating them with IgE having the desired antigenic specificity, resulting in the release of soluble mediators such as histamine and leukotrienes when induced [117]. A reported study by Jin et al. (2019) has also induced these cells using PMA (50 nM) and A23187 (1 μM) for 24 h to measure the release of histamine and LTC4 levels [118]. P815 cells, on the other hand were isolated from a mouse with mastocytoma in 1957[118,119]. These cells do not express the IgE high affinity receptor and, therefore, can only be activated independent of the IgE pathway. However, they do express fragment crystallizable gamma receptor II (FcγRII) and could bind the Fc portion of mouse IgG antibodies through their fragment antigen-binding (Fab) which may recognize NK cells activating receptors leading to target cell lysis[120]. In addition, one study reported the use of C48/80 and CI to induce degranulation in P815 cells to release allergic mediators such as IL-4 and histamine [121].

7.0 Conclusion

Using secondary mast cell lines to conduct research in allergy has come a long way since the discovery of mast cells in 1863[122]. Today in modern research, the scientific community is able to utilize different mast cell lines to study the role of mast cell in an allergic response. This systematic review illustrated the popular secondary mast cell lines that are used to study the molecular and cellular mechanisms involved in functional studies in allergy diseases, and to determine potential protective compounds for allergic treatments (Figure 5). Therefore, some of these well-known cell lines such as RBL-2H3, HMC-1, and LAD2 cells have been proven to be an asset in unraveling the molecular complexity of allergy. However, every cell line will have its own drawbacks such as absence of high affinity IgE receptor and long doubling time. In addition, the differences in cell source and maintenance in culture may influence the epigenetic character of these cells which potentially lead to results variations and inconsistency across laboratories. Thus, researchers must take into consideration of these factors before selecting the appropriate mast cell line for their studies.

8.0 Availability of data and material

Data available on request from the authors.

9.0 Author Contributions

YZL, ASFK, CSAW, and AJWY performed database searching, article screening, study selection and appraisal, and data extraction and analysis. JWT, YZL, ASFK, CSAW, and AJWY conceived the idea and wrote the manuscript. JWT supervised the study and resolved conflicts between reviewers (YZL, ASFK, CSAW, and AJWY). CLT and MTL provided important information for the completion of the manuscript. All authors read and approved the final manuscript.

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12.0 Conflict of Interest

The Authors have declared that there are no conflicts of interest.

13.0 Consent for publication

The manuscript has been read and approved for publication by all the named authors.

14.0 References

1 Igea JM. The history of the idea of allergy. *Allergy* 2013;**68** : 966–973.

2 Aldakheel FM. Allergic Diseases: A Comprehensive Review on Risk Factors, Immunological Mechanisms, Link with COVID-19, Potential Treatments, and Role of Allergen Bioinformatics. *IJERPH* 2021;**18** : 12105.

3 Choi HW, Abraham SN. In Vitro and In Vivo IgE-/Antigen-Mediated Mast Cell Activation. In: Reinhardt RL (ed). *Type 2 Immunity* . Springer New York: New York, NY, 2018, pp 71–80.

4 Moote W, Kim H, Ellis AK. Allergen-specific immunotherapy.*Allergy Asthma Clin Immunol* 2018; **14** : 53.

5 Schoos A-MM, Bullens D, Chawes BL, Costa J, De Vlieger L, DunnGalvin A*et al.* Immunological Outcomes of Allergen-Specific Immunotherapy in Food Allergy. *Front Immunol* 2020; **11** : 568598.

6 Kanagaratham C, El Ansari YS, Lewis OL, Oettgen HC. IgE and IgG Antibodies as Regulators of Mast Cell and Basophil Functions in Food Allergy. *Front Immunol* 2020; **11** : 603050.

7 Wang N, Wang J, Zhang Y, Zeng Y, Hu S, Bai H *et al.* Imperatorin ameliorates mast cell-mediated allergic airway inflammation by inhibiting MRGPRX2 and CamKII/ERK signaling pathway. *Biochemical Pharmacology* 2021; **184** : 114401.

8 Brown JM, Wilson TM, Metcalfe DD. The mast cell and allergic diseases: role in pathogenesis and implications for therapy. *Clin Experimental Allergy* 2008; **38** : 4–18.

9 Hermans MAW, Van Stigt AC, Van De Meerendonk S, Schrijver B, Van Daele PLA, Van Hagen PM *et al.* Human Mast Cell Line HMC1 Expresses Functional Mas-Related G-Protein Coupled Receptor 2. *Front Immunol* 2021; **12** : 625284.

10 L'homme L, Dombrowicz D. In vitro models of human mast cells: How to get more and better with induced pluripotent stem cells? *Journal of Allergy and Clinical Immunology* 2022; **149** : 904–906.

11 Huang J, Liu C, Wang Y, Wang C, Xie M, Qian Y *et al.*Application of in vitro and in vivo models in the study of food allergy.*Food Science and Human Wellness* 2018; **7** : 235–243.

12 Butterfield JH, Weiler D, Dewald G, Gleich GJ. Establishment of an immature mast cell line from a patient with mast cell leukemia.*Leukemia Research* 1988; **12** : 345–355.

13 Valent P, Bettelheim P. Cell Surface Structures on Human Basophils and Mast Cells: Biochemical and Functional Characterization. In:*Advances in Immunology* . Elsevier, 1992, pp 333–423.

14 Nilsson G, Blom T, Kusche-Gullberg M, Kjellen L, Butterfield JH, Sundström C *et al.* Phenotypic Characterization of the Human Mast-Cell Line HMC-1. *Scand J Immunol* 1994; **39** : 489–498.

15 Sundstrom M, Vliagoftis H, Karlberg P, Butterfield JH, Nilsson K, Metcalfe DD *et al.* Functional and phenotypic studies of two variants of a human mast cell line with a distinct set of mutations in the *c-kit* proto-oncogene. *Immunology* 2003; **108** : 89–97.

16 Tobio A, Alfonso A, Botana LM. Cross-talks between c-Kit and PKC isoforms in HMC-1560 and HMC-1560,816 cells. Different role of PKCδ in each cellular line. *Cellular Immunology* 2015; **293** : 104–112.

17 Furitsu T, Tsujimura T, Tono T, Ikeda H, Kitayama H, Koshimizu U*et al.* Identification of mutations in the coding sequence of the proto-oncogene c-kit in a human mast cell leukemia cell line causing ligand-independent activation of c-kit product. *J Clin Invest*1993; **92** : 1736–1744.

18 Da Silva CA, Reber L, Frossard N. Stem cell factor expression, mast cells and inflammation in asthma. *Fundamemntal Clinical Pharma*2006; **20** : 21–39.

19 Rådinger M, Jensen BM, Kuehn HS, Kirshenbaum A, Gilfillan AM. Generation, Isolation, and Maintenance of Human Mast Cells and Mast Cell Lines Derived from Peripheral Blood or Cord Blood. *CP in Immunology* 2010; **90** . doi:10.1002/0471142735.im0737s90.

20 Ma Y, Zeng S, Metcalfe DD, Akin C, Dimitrijevic S, Butterfield JH*et al.* The c-KIT mutation causing human mastocytosis is resistant to STI571 and other KIT kinase inhibitors; kinases with enzymatic site mutations show different inhibitor sensitivity profiles than wild-type kinases and those with regulatory-type mutations. *Blood* 2002;**99** : 1741–1744.

21 Sun J, Pedersen M, Rönnstrand L. The D816V Mutation of c-Kit Circumvents a Requirement for Src Family Kinases in c-Kit Signal Transduction. *Journal of Biological Chemistry* 2009; **284** : 11039–11047.

22 Guhl S, Babina M, Neou A, Zuberbier T, Artuc M. Mast cell lines HMC-1 and LAD2 in comparison with mature human skin mast cells – drastically reduced levels of tryptase and chymase in mast cell lines.*Experimental Dermatology* 2010; **19** : 845–847.

23 Krystel-Whittemore M, Dileepan KN, Wood JG. Mast Cell: A Multi-Functional Master Cell. *Front Immunol* 2016; **6** . doi:10.3389/fimmu.2015.00620.

24 Lee M, Lee NY, Chung K-S, Cheon S-Y, Lee K-T, An H-J. Roxatidine attenuates mast cell-mediated allergic inflammation via inhibition of NF-κB and p38 MAPK activation. *Sci Rep* 2017; **7** : 41721.

25 Wu X, Lin M, Li Y, Zhao X, Yan F. Effects of DMEM and RPMI 1640 on the biological behavior of dog periosteum-derived cells.*Cytotechnology* 2009; **59** : 103–111.

26 Huang Z, Shao W, Gu J, Hu X, Shi Y, Xu W *et al.* Effects of culture media on metabolic profiling of the human gastric cancer cell line SGC7901. *Mol BioSyst* 2015; **11** : 1832–1840.

27 Liu YK, Lu QZ, Pei R, Ji HJ, Zhou GS, Zhao XL *et al.* The effect of extracellular calcium and inorganic phosphate on the growth and osteogenic differentiation of mesenchymal stem cells *in vitro* : implication for bone tissue engineering. *Biomed Mater*2009; **4** : 025004.

28 Afzan MY, Mohammed AJ. Role of L-glutamine in the in-vitro growth of HCT-8 and HT-29 cell lines. *Mal J Anim* 2019; **23** : 580–585.

29 Jagušić M, Forčić D, Brgles M, Kutle L, Šantak M, Jergović M *et al.* Stability of Minimum Essential Medium functionality despite l-glutamine decomposition. *Cytotechnology* 2016; **68** : 1171–1183.

30 Coëffier M, Miralles-Barrachina O, Le Pessot F, Lalaude O, Daveau M, Lavoinne A *et al.* INFLUENCE OF GLUTAMINE ON CYTOKINE PRODUCTION BY HUMAN GUT IN VITRO. *Cytokine* 2001; **13** : 148–154.

31 Lechowski S, Feilhauer K, Staib L, Coëffier M, Bischoff SC, Lorentz A. Combined arginine and glutamine decrease release of de novo synthesized leukotrienes and expression of proinflammatory cytokines in activated human intestinal mast cells. *Eur J Nutr* 2013;**52** : 505–512.

32 Wang X, Huang L, Qu Y, Lv H, He X. Effects of glutamine on cytokines 1L-1 and TNF-α in rehabilitation and prognosis of patients with lobectomy. *Exp Ther Med* 2018. doi:10.3892/etm.2018.6443.

33 Horio Y, Osawa S, Takagaki K, Hishida A, Furuta T, Ikuma M. Glutamine supplementation increases Th1-cytokine responses in murine intestinal intraepithelial lymphocytes. *Cytokine* 2008; **44** : 92–95.

34 Abusalamah H, Reel JM, Lupfer CR. Pyruvate affects inflammatory responses of macrophages during influenza A virus infection. *Virus Research* 2020; **286** : 198088.

35 Nitta N, Aoki Y, Isogawa Y, Tsuchiya T, Kanegasaki S. Image analysis of mast cell degranulation in a concentration gradient of stimuli formed in the channel between a glass plate and a silicon substrate.*European Journal of Cell Biology* 2009; **88** : 541–549.

36 Chelombitko MA, Firsov AM, Kotova EA, Rokitskaya TI, Khailova LS, Popova LB *et al.* Usnic acid as calcium ionophore and mast cells stimulator. *Biochimica et Biophysica Acta (BBA) - Biomembranes*2020; **1862** : 183303.

37 Pi J, Huang L, Yang F, Jiang J, Jin H, Liu J *et al.* Atomic force microscopy study of ionomycin-induced degranulation in RBL-2H3 cells. *Scanning* 2016; **38** : 525–534.

38 Chang SN, Dey DK, Oh ST, Kong WH, Cho KH, Al-Olayan EM *et al.*Phorbol 12-Myristate 13-Acetate Induced Toxicity Study and the Role of Tangeretin in Abrogating HIF-1α-NF-ϰB Crosstalk In Vitro and In Vivo.*IJMS* 2020; **21** : 9261.

39 Keski-Nisula L, Roponen M, Hirvonen M-R, Heinonen S, Pekkanen J. Stimulated cytokine production correlates in umbilical arterial and venous blood at delivery. *Eur Cytokine Netw* 2004; **15** : 347–352.

40 Barten MJ, Rahmel A, Bocsi J, Boldt A, Garbade J, Dhein S *et al.* Cytokine analysis to predict immunosuppression. *Cytometry Pt A* 2006; **69A** : 155–157.

41 Olsen I, Sollid LM. Pitfalls in determining the cytokine profile of human T cells. *Journal of Immunological Methods* 2013;**390** : 106–112.

42 Zimmermann J, Radbruch A, Chang H. A Ca^{2+}concentration of 1.5 mM, as present in IMDM but not in RPMI, is critical for maximal response of Th cells to PMA/ionomycin. *Eur J Immunol*2015; **45** : 1270–1273.

43 Ai W, Li H, Song N, Li L, Chen H. Optimal Method to Stimulate Cytokine Production and Its Use in Immunotoxicity Assessment.*IJERPH* 2013; **10** : 3834–3842.

44 Schemann M, Kugler EM, Buhner S, Eastwood C, Donovan J, Jiang W*et al.* The Mast Cell Degranulator Compound 48/80 Directly Activates Neurons. *PLoS ONE* 2012; **7** : e52104.

45 Kirshenbaum AS, Akin C, Wu Y, Rottem M, Goff JP, Beaven MA *et al.* Characterization of novel stem cell factor responsive human mast cell lines LAD 1 and 2 established from a patient with mast cell sarcoma/leukemia; activation following aggregation of FcεRI or FcγRI.*Leukemia Research* 2003; **27** : 677–682.

46 Kirshenbaum AS, Yin Y, Sundstrom JB, Bandara G, D. Metcalfe D. Description and Characterization of a Novel Human Mast Cell Line for Scientific Study. *IJMS* 2019; **20** : 5520.

47 Kirshenbaum AS, Petrik A, Walsh R, Kirby TL, Vepa S, Wangsa D*et al.* A Ten-Year Retrospective Analysis of the Distribution, Use and Phenotypic Characteristics of the LAD2 Human Mast Cell Line.*Int Arch Allergy Immunol* 2014; **164** : 265–270.

48 Ohta Y, Yashiro K, Ohashi K, Horikoshi Y, Kusumoto C, Matsura T. Compound 48/80, a mast cell degranulator, causes oxidative damage by enhancing vitamin C synthesis via reduced glutathione depletion and lipid peroxidation through neutrophil infiltration in rat livers.*J Clin Biochem Nutr* 2017; **60** : 187–198.

49 Che D, Zheng Y, Hou Y, Du X, Jia T, Zhao Q *et al.* Action of substance P and PAMP(9-20) on different excitation sites of MRGPRX2 induces differences in mast cell activation. *International Immunopharmacology* 2021; **101** : 108342.

50 Wolf K, Kühn H, Boehm F, Gebhardt L, Glaudo M, Agelopoulos K *et al.* A group of cationic amphiphilic drugs activates MRGPRX2 and induces scratching behavior in mice. *Journal of Allergy and Clinical Immunology* 2021; **148** : 506-522.e8.

51 Che D, Gao J, Du X, Zheng Y, Hou Y, Peng B *et al.*p-Phenylenediamine induces immediate contact allergy and non-histaminergic itch via MRGPRX2. *Chemico-Biological Interactions* 2022; **351** : 109751.

52 Duraisamy K, Singh K, Kumar M, Lefranc B, Bonnafé E, Treilhou M*et al.* P17 induces chemotaxis and differentiation of monocytes via MRGPRX2-mediated mast cell–line activation. *Journal of Allergy and Clinical Immunology* 2022; **149** : 275–291.

53 Choi HW, Chan C, Shterev ID, Lynch HE, Robinette TJ, Johnson-Weaver BT *et al.* Identification of Novel Mast Cell Activators Using Cell-Based High-Throughput Screening. *SLAS Discovery* 2019;**24** : 628–640.

54 Ng K, Raheem J, St Laurent CD, Marcet CT, Vliagoftis H, Befus AD*et al.* Responses of human mast cells and epithelial cells following exposure to influenza A virus. *Antiviral Research* 2019;**171** : 104566.

55 Lian Q, Cheng Y, Zhong C, Wang F. Inhibition of the IgE-Mediated Activation of RBL-2H3 Cells by TIPP, a Novel Thymic Immunosuppressive Pentapeptide. *IJMS* 2015; **16** : 2252–2268.

56 Lee B-C, Kim J-J, Lee JY, Kang I, Shin N, Lee S-E *et al.*Disease-specific primed human adult stem cells effectively ameliorate experimental atopic dermatitis in mice. *Theranostics* 2019;**9** : 3608–3621.

57 Park YH, Kim DK, Kim HS, Lee D, Lee MB, Min KY *et al.* WZ3146 inhibits mast cell Lyn and Fyn to reduce IgE-mediated allergic responses in vitro and in vivo. *Toxicology and Applied Pharmacology* 2019;**383** : 114763.

58 Sun M, Qin D, Fan P, Chen X, Liu Y. Chitosan-centered nanosystems as sustained therapeutics for allergic rhinitis intervention: Inhibition of histamine-induced cascades. *Journal of Controlled Release* 2021;**335** : 422–436.

59 Falcone FH, Wan D, Barwary N, Sagi-Eisenberg R. RBL cells as models for in vitro studies of mast cells and basophils.*Immunological Reviews* 2018; **282** : 47–57.

60 Eccleston E, Leonard BJ, Lowe JS, Welford HJ. Basophilic Leukaemia in the Albino Rat and a Demonstration of the Basopoietin. *Nature New Biology* 1973; **244** : 73–76.

61 Kulczycki A, Metzger H. The Interaction of IgE with Rat Basophilic Leukemia Cells. *The Journal of Experimental Medicine* 1974;**140** : 1676–1695.

62 Kulczycki A, Isersky C, Metzger H. The Interaction of IgE with Rat Basophilic Leukemia Cells. *The Journal of Experimental Medicine*1974; **139** : 600–616.

63 Siraganian RP, Kulczycki A, Mendoza G, Metzger H. Ionophore A-23187 Induced Histamine Release from Rat Mast Cells and Rat Basophil Leukemia (RBL-1) Cells. *The Journal of Immunology* 1975; **115** : 1599–1602.

64 Barsumian EL, Isersky C, Petrino MG, Siraganian RP. IgE-induced histamine release from rat basophilic leukemia cell lines: isolation of releasing and nonreleasing clones. *Eur J Immunol* 1981;**11** : 317–323.

65 Carson DA, Kulczycki A, Metzger H. Interaction of IgE with Rat Basophilic Leukemia Cells. *The Journal of Immunology* 1975;**114** : 158–160.

66 Passante E, Frankish N. The RBL-2H3 cell line: its provenance and suitability as a model for the mast cell. *Inflamm Res* 2009;**58** : 737–745.

67 Passante E, Ehrhardt C, Sheridan H, Frankish N. RBL-2H3 cells are an imprecise model for mast cell mediator release. *Inflamm Res* 2009;**58** : 611–618.

68 Mazurek N, Bashkin P, Petrank A, Pecht I. Basophil variants with impaired cromoglycate binding do not respond to an immunological degranulation stimulus. *Nature* 1983; **303** : 528–530.

69 Gibbs BF, Amon FL, Pearce U. Spontaneous histamine release from mast cells and basophils is controlled by the cellular environment.*Inflammation Research* 1997; **46** : 25–26.

70 Boyce JA, Friend D, Matsumoto R, Austen KF, Owen WF. Differentiation in vitro of hybrid eosinophil/basophil granulocytes: autocrine function of an eosinophil developmental intermediate. *The Journal of experimental medicine* 1995; **182** : 49–57.

71 Kirshenbaum AS, Goff JP, Semere T, Foster B, Scott LM, Metcalfe DD. Demonstration That Human Mast Cells Arise From a Progenitor Cell Population That Is CD34+, c-kit+, and Expresses Aminopeptidase N (CD13).*Blood* 1999; **94** : 2333–2342.

72 Denburg J. Basophil and mast cell lineages in vitro and in vivo [published erratum appears in Blood 1992 Aug 1;80(3):855].*Blood* 1992; **79** : 846–860.

73 Li L, Li Y, Reddel SW, Cherrian M, Friend DS, Stevens RL *et al.* Identification of basophilic cells that express mast cell granule proteases in the peripheral blood of asthma, allergy, and drug-reactive patients. *J Immunol* 1998; **161** : 5079–5086.

74 Maeyama K, Hohman RJ, Metzger H, Beaven MA. Quantitative relationships between aggregation of IgE receptors, generation of intracellular signals, and histamine secretion in rat basophilic leukemia (2H3) cells. Enhanced responses with heavy water. *J Biol Chem* 1986; **261** : 2583–2592.

75 Seldin DC, Adelman S, Austen KF, Stevens RL, Hein A, Caulfield JP*et al.* Homology of the rat basophilic leukemia cell and the rat mucosal mast cell. *Proc Natl Acad Sci USA* 1985; **82** : 3871–3875.

76 Tsujimura T, Furitsu T, Morimoto M, Kanayama Y, Nomura S, Matsuzawa Y*et al.* Substitution of an Aspartic Acid Results in Constitutive Activation of c-*kit* Receptor Tyrosine Kinase in a Rat Tumor Mast Cell Line RBL-2H3. *Int Arch Allergy Immunol* 1995; **106** : 377–385.

77 Gilfillan AM, Tkaczyk C. Integrated signalling pathways for mast-cell activation. *Nat Rev Immunol* 2006; **6** : 218–230.

78 Coleman JW. A kinetic analysis of the in vitro sensitization of murine peritoneal mast cells with monoclonal IgE anti-DNP antibody.*Immunology* 1988; **64** : 527–531.

79 Dearman R, Skinner R, Deakin N, Shaw D, Kimber I. Evaluation of an in vitro method for the measurement of specific IgE antibody responses: the rat basophilic leukemia (RBL) cell assay. *Toxicology* 2005;**206** : 195–205.

80 Moon TC, Befus AD, Kulka M. Mast Cell Mediators: Their Differential Release and the Secretory Pathways Involved. *Front Immunol* 2014;**5** . doi:10.3389/fimmu.2014.00569.

81 Dedkova EN, Sigova AA, Zinchenko VP. Mechanism of action of calcium ionophores on intact cells: ionophore-resistant cells. *Membr Cell Biol* 2000; **13** : 357–368.

82 Johnson HG, Bach MK. Prevention of Calcium Ionophore-Induced Release of Histamine in Rat Mast Cells by Disodium Cromoglycate. *The Journal of Immunology* 1975; **114** : 514–516.

83 Ludowyke RI, Scurr LL, McNally CM. Calcium ionophore-induced secretion from mast cells correlates with myosin light chain phosphorylation by protein kinase C. *The Journal of Immunology*1996; **157** : 5130–5138.

84 Mousli M, Bueb J-L, Bronner C, Rouot B, Landry Y. G protein activation: a receptor-independent mode of action for cationic amphiphilic neuropeptides and venom peptides. *Trends in Pharmacological Sciences* 1990; **11** : 358–362.

85 Tomita U, Inanobe A, Kobayashi I, Takahashi K, Ui M, Katada T. Direct Interactions of Mastoparan and Compound 48/80 with GTP-Binding Proteins1. *The Journal of Biochemistry* 1991; **109** : 184–189.

86 Tanaka T, Kohno T, Kinoshita S, Mukai H, Itoh H, Ohya M *et al.*α Helix Content of G Protein α Subunit Is Decreased upon Activation by Receptor Mimetics. *Journal of Biological Chemistry* 1998;**273** : 3247–3252.

87 Senyshyn J, Baumgartner RA, Beaven MA. Quercetin Sensitizes RBL-2H3 Cells to Polybasic Mast Cell Secretagogues Through Increased Expression of Gi GTP-Binding Proteins Linked to a Phospholipase C Signaling Pathway. *The Journal of Immunology* 1998; **160** : 5136–5144.

88 Chahdi A, Fraundorfer PF, Beaven MA. Compound 48/80 activates mast cell phospholipase D via heterotrimeric GTP-binding proteins. *J Pharmacol Exp Ther* 2000; **292** : 122–130.

89 Swieter M, Midura RJ, Nishikata H, Oliver C, Berenstein EH, Mergenhagen SE *et al.* Mouse 3T3 fibroblasts induce rat basophilic leukemia (RBL-2H3) cells to acquire responsiveness to compound 48/80. *The Journal of Immunology* 1993; **150** : 617–624.

90 Hasala H, Giembycz MA, Janka-Junttila M, Moilanen E, Kankaanranta H. Histamine reverses IL-5-afforded human eosinophil survival by inducing apoptosis: Pharmacological evidence for a novel mechanism of action of histamine. *Pulmonary Pharmacology & Therapeutics* 2008;**21** : 222–233.

91 Nakatani K, Atsumi M, Arakawa T, Oosawa K, Shimura S, Nakahata N*et al.* Inhibitions of Histamine Release and Prostaglandin E2 Synthesis by Mangosteen, a Thai Medicinal Plant. *Biological & Pharmaceutical Bulletin* 2002; **25** : 1137–1141.

92 Kobayashi M, Matsushita H, Yoshida K, Tsukiyama R-I, Sugimura T, Yamamoto K. In vitro and in vivo anti-allergic activity of soy sauce.*Int J Mol Med* 2004. doi:10.3892/ijmm.14.5.879.

93 Marshall JS. Mast-cell responses to pathogens. *Nat Rev Immunol*2004; **4** : 787–799.

94 Gon Y, Nunomura S, Ra C. Common and distinct signalling cascades in the production of tumour necrosis factor-α and interleukin-13 induced by lipopolysaccharide in RBL-2H3 cells. *Clin Experimental Allergy*2005; **35** : 635–642.

95 Conrad DH, Wingard JR, Ishizaka T. The interaction of human and rodent IgE with the human basophil IgE receptor. *The Journal of Immunology* 1983; **130** : 327–333.

96 Hakimi J, Seals C, Kondas JA, Pettine L, Danho W, Kochan J. The alpha subunit of the human IgE receptor (FcERI) is sufficient for high affinity IgE binding. *J Biol Chem* 1990; **265** : 22079–22081.

97 Gilfillan AM, Kado-Fong H, Wiggan GA, Hakimi J, Kent U, Kochan JP. Conservation of signal transduction mechanisms via the human Fc epsilon RI alpha after transfection into a rat mast cell line, RBL 2H3. *J Immunol* 1992; **149** : 2445–2451.

98 Taudou G, Varin-Blank N, Jouin H, Marchand F, Weyer A, Blank U. Expression of the Alpha Chain of Human FcεRI in Transfected Rat Basophilic Leukemia Cells: Functional Activation after Sensitization with Human Mite-Specific IgE. *Int Arch Allergy Immunol* 1993;**100** : 344–350.

99 Wilson APM, Pullar CE, Camp AM, Helm BA. Human IgE mediates stimulus secretion coupling in rat basophilic leukemia cells transfected with the α chain of the human high-affinity receptor. *Eur J Immunol* 1993;**23** : 240–244.

100 Vogel L, Lüttkopf D, Hatahet L, Haustein D, Vieths S. Development of a functional *in vitro* assay as a novel tool for the standardization of allergen extracts in the human system. *Allergy*2005; **60** : 1021–1028.

101 Lowe J, Jardieu P, VanGorp K, Fei DTW. Allergen-induced histamine release in rat mast cells transfected with the α subunits of FcεRI. *Journal of Immunological Methods* 1995; **184** : 113–122.

102 Ladics GS, Van Bilsen JHM, Brouwer HMH, Vogel L, Vieths S, Knippels LMJ. Assessment of three human FcεRI-transfected RBL cell-lines for identifying IgE induced degranulation utilizing peanut-allergic patient sera and peanut protein extract. *Regulatory Toxicology and Pharmacology* 2008; **51** : 288–294.

103 Blanc F, Adel-Patient K, Drumare M -F., Paty E, Wal J -M., Bernard H. Capacity of purified peanut allergens to induce degranulation in a functional *in vitro* assay: Ara h 2 and Ara h 6 are the most efficient elicitors. *Clin Experimental Allergy* 2009; **39** : 1277–1285.

104 Sun N, Zhou C, Zhou X, Sun L, Che H. Use of a rat basophil leukemia (RBL) cell-based immunological assay for allergen identification, clinical diagnosis of allergy, and identification of anti-allergy agents for use in immunotherapy. *Journal of Immunotoxicology* 2015;**12** : 199–205.

105 Wiegand TW, Williams PB, Dreskin SC, Jouvin MH, Kinet JP, Tasset D. High-affinity oligonucleotide ligands to human IgE inhibit binding to Fc epsilon receptor I. *The Journal of Immunology* 1996; **157** : 221–230.

106 Nakamura R, Uchida Y, Higuchi M, Nakamura R, Tsuge I, Urisu A *et al.* A convenient and sensitive allergy test: IgE crosslinking-induced luciferase expression in cultured mast cells. *Allergy* 2010; **65** : 1266–1273.

107 Hogan PG, Chen L, Nardone J, Rao A. Transcriptional regulation by calcium, calcineurin, and NFAT. *Genes Dev* 2003; **17** : 2205–2232.

108 Wan D, Wang X, Nakamura R, Alcocer MJC, Falcone FH. Use of Humanized RBL Reporter Systems for the Detection of Allergen-Specific IgE Sensitization in Human Serum. In: Gibbs BF, Falcone FH (eds). *Basophils and Mast Cells* . Springer US: New York, NY, 2020, pp 145–153.

109 Wan D, Ludolf F, Alanine DGW, Stretton O, Ali Ali E, Al-Barwary N *et al.* Use of Humanised Rat Basophilic Leukaemia Cell Line RS-ATL8 for the Assessment of Allergenicity of Schistosoma mansoni Proteins. *PLoS Negl Trop Dis* 2014; **8** : e3124.

110 Falcone FH, Alcocer MJC, Okamoto-Uchida Y, Nakamura R. Use of Humanized Rat Basophilic Leukemia Reporter Cell Lines as a Diagnostic Tool for Detection of Allergen-Specific IgE in Allergic Patients: Time for a Reappraisal? *Curr Allergy Asthma Rep* 2015; **15** : 67.

111 Lupi R, Denery-Papini S, Claude M, Tranquet O, Drouet M, Masci S *et al.* Thermal treatment reduces gliadin recognition by IgE, but a subsequent digestion and epithelial crossing permits recovery. *Food Research International* 2019; **118** : 22–31.

112 Hazebrouck S, Patil SU, Guillon B, Lahood N, Dreskin SC, Adel-Patient K *et al.* Immunodominant conformational and linear IgE epitopes lie in a single segment of Ara h 2. *Journal of Allergy and Clinical Immunology* 2022; **150** : 131–139.

113 Villazala-Merino S, Rodriguez-Dominguez A, Stanek V, Campion NJ, Gattinger P, Hofer G *et al.* Allergen-specific IgE levels and the ability of IgE-allergen complexes to cross-link determine the extent of CD23-mediated T-cell activation. *Journal of Allergy and Clinical Immunology* 2020; **145** : 958-967.e5.

114 Laidlaw TM, Steinke JW, Tiñana AM, Feng C, Xing W, Lam BK *et al.* Characterization of a novel human mast cell line that responds to stem cell factor and expresses functional FcεRI. *Journal of Allergy and Clinical Immunology* 2011; **127** : 815-822.e5.

115 Saleh R, Wedeh G, Herrmann H, Bibi S, Cerny-Reiterer S, Sadovnik I *et al.* A new human mast cell line expressing a functional IgE receptor converts to tumorigenic growth by KIT D816V transfection. *Blood* 2014; **124** : 111–120.

116 Galli SJ, Dvorak AM, Marcum JA, Ishizaka T, Nabel G, Der Simonian H*et al.* Mast cell clones: a model for the analysis of cellular maturation. *The Journal of cell biology* 1982; **95** : 435–444.

117 Staats HF, Kirwan SM, Choi HW, Shelburne CP, Abraham SN, Leung GYC*et al.* A mast cell degranulation screening assay for the identification of novel mast cell activating agents. *Med Chem Commun* 2013; **4** : 88–94.

118 Jin S, Ha H, Shin H-K, Seo C-S. Anti-Allergic and Anti-Inflammatory Effects of Kuwanon G and Morusin on MC/9 Mast Cells and HaCaT Keratinocytes. *Molecules* 2019; **24** : 265.

119 Gajewski TF, Markiewicz MA, Uyttenhove C. The P815 Mastocytoma Tumor Model. *CP in Immunology* 2001; **43** . doi:10.1002/0471142735.im2004s43.

120 Terrén I, Astarloa-Pando G, Amarilla-Irusta A, Borrego F. P815-based redirected degranulation assay to study human NK cell effector functions. In: *Methods in Cell Biology* . Elsevier, 2023, pp 33–48.

121 Chen B-R, Hsu K-T, Hsu W-H, Lee B-H, Li T-L, Chan Y-L *et al.*Immunomodulation and mechanisms of fucoidan from Cladosiphon okamuranus ameliorates atopic dermatitis symptoms. *International Journal of Biological Macromolecules* 2021; **189** : 537–543.

122 Blank U, Falcone FH, Nilsson G. The history of mast cell and basophil research – some lessons learnt from the last century.*Allergy* 2013; **68** : 1093–1101.

Table 1. Detailed information on the type of basal media, supplements used, and their respective number of articles for culturing HMC-1 cells.

Basal Media		**Supplements**			
		Serum		Others	
Name	# articles	Name	# articles	Name	# articles
IMDM	43	10% FBS	45	Penicillin/streptomycin	44
RPMI-1640	5	10% FCS	2	L-glutamine	4
DMEM	1			Monothioglycerol	3
IMEM	1			α-thioglycerol	2
				2-mercaptoethanol	1
				Amphotericin B	1
				Sodium bicarbonate	1

IMDM: Iscove's Modified Dulbecco's Medium; RPMI-1640: Roswell Park Memorial Institute-1640; DMEM: Dulbecco's Modified Eagle Medium; IMEM: Improved Minimum Essential Media; FBS: Fetal Bovine Serum.

Table 2. Detailed information on the number of articles for the type of inducers, their induction time, and the type of mediators studied in HMC-1 cells activation.

Type of inducers		Induction		Type of mediators	
Name	# articles	Time	# articles	Name	# articles
PMA	25	<1h	20	TNF-α	29
CI	28	2-4h	13	β-hexosaminidase	7
Compound 48/80	3	5-8h	23	histamine	17
OVA	3	9-12h	2	IL-1β	20
IL-33	3	13-24h	16	IL-1RA	1
TSLP	3	Unknown	4	IL-2	2
Protein kinase C activator	2			IL-4	7
RANKL	2			IL-5	4
LPS	2			IL-6	27
histamine	2			IL-7RA	1
DNP-HAS	1			IL-8	12
EGCG	1			IL-10	7
IgD	1			IL-13	6
IgE	1			IL-17	2
PFC	1			IL-18	2
BPA	1			IL-21	1
DMSO	1			IL-23	1
bisdemethoxycurcumin	1			IL-31	1
thrombin	1			IL-33	1
Par1 agonist	1			IFN-γ	3
Par4 agonist	1			IgE	1
Par1 inhibitor	1			IgG1	1
Par4 inhibitor	1			IgG2a	1
ERK1/2 inhibitor	1			LTB4	2
P38 inhibitor	1			LTC4	1
JNK inhibitor	1			PGD2	1
Lactococcus lactis	1			TSLP	14
Unknown	3			RANTES	1
				VEGF	1
				TGFβ1	1
				CXCL 10	1
				Tryptase	3
				Caspase 1	9
				Calcium ions	6

PMA: phorbol 12-myristate 13-acetate; CI: calcium ionophore; OVA: ovalbumin; TSLP: thymic stromal lymphopoietin; RANKL: receptor activator of NF-κβ ligand; LPS: lipopolysaccharide; DNP: 2, 4-Dinitrophenol; EGCG: epigallocatechin gallate; PFC: perfluoroalkyl compounds; BPA: bisphenol A; DMSO: dimethylsulfoxide; TNF: tumor necrosis factor; RA: receptor antagonist; LTB4: leukotriene B4; LTC4: leukotriene C4; PGD2: prostaglandin D2; RANTES: regulated upon activation, normal T cell expressed and secreted; VEGF: vascular endothelial growth factor; CXCL: chemokine (C-X-C motif) ligand 1; ND: No data.

Table 3. Detailed information on the type of basal media, supplements used, and their respective number of articles for culturing LAD2 cells.

Basal Media		Supplements			
		Serum		Others	
Name	# articles	Name	# articles	Name	# articles
StemPro-34	73	10% FCS	1	StemPro-34 nutrient	73
IMDM	1				

Table 4. Detailed information on the number of articles for the type of inducers, their induction time, and the type of mediators studied in LAD2 cells activation

Type inducers		**Induction**		**Type of mediators**	
Name	# articles	Time	# articles	Name	# articles
Substance P	14	<1h	60	β-hexosaminidase	62
Tween 20	1	2-4h	6	Histamine	36
PAMP(9-20)	3	5-8h	30	5-HT	3
PAMP-12	1	13-24h	13	Tryptase	6
Compound 48/80	36	2-4 days	3	CD63	3
LL-37	4	Unknown	5	TNF-α	28
(R)-ZINC-3573	3			IL-1α	1
CST-14	1			IL-1RA	1
Thapsigargin	1			IL-1β	2
Complement C3a	3			IL-2	1
Antidepressants	1			IL-4	3
Gadopentetate meglumine	1			IL-5	2
Iodinated contrast media	1			IL-6	5
p-Phenylenediamine	1			IL-8	27
P17	1			IL-10	2
Morphine derivatives	1			IL-13	5
IL-17A	1			IL-17	1
IL-33	3			IL-17A	2
FluA virus and UV inactivated-FluA	1			IL-22	1
Novel mast cell compounds	1			IL-31	1
Mastoparan	1			IL-33	1
PMA and Ionomycin	2			MCP-1	30
PMACI	1			CCL-1	1
Thimerosol	1			CCL-2	25
Iopamidol	1			CCL-3	2
Isosalvianolic acid C	1			CCL-4	3
Fluoroquinolones	1			CCL-5	3
Polymycin B and Polymycin E	1			MIP-1α	2
Microvesicles from activated T cells	1			MIP-1β	3
SQ21 or SQ22	1			PGD2	6
Nanoparticle formulations	1			LTB4	1
Imidazolidinyl urea	1			GM-CSF	3
A23187	3			G-CSF	1
Biotin-conjugated IgE	1			Flt-3L	1
Water	1			CXCL-10	1
DNP-BSA	3			IFN-γ	2
DNP-HSA	4			FGF2	1
DNP-Streptomycin	1			VEGF	1
Streptavidin	11			CysLTs	1
human Astressin 2B	1				
WZ3146/PP2	1				
Anti-IgE	5				
NECA	1				
Milk particle	1				
IFN-β1	1				
Polypeptide	1				
Frozen and thawed 3 times	1				

NECA: 5'-N'(ethylcarboxamido)adenosine; CysLTs: Cysteinyl leukotrienes

Table 5 Detailed information on the type of basal media, supplements used, and their respective number of articles for culturing RBL-2H3

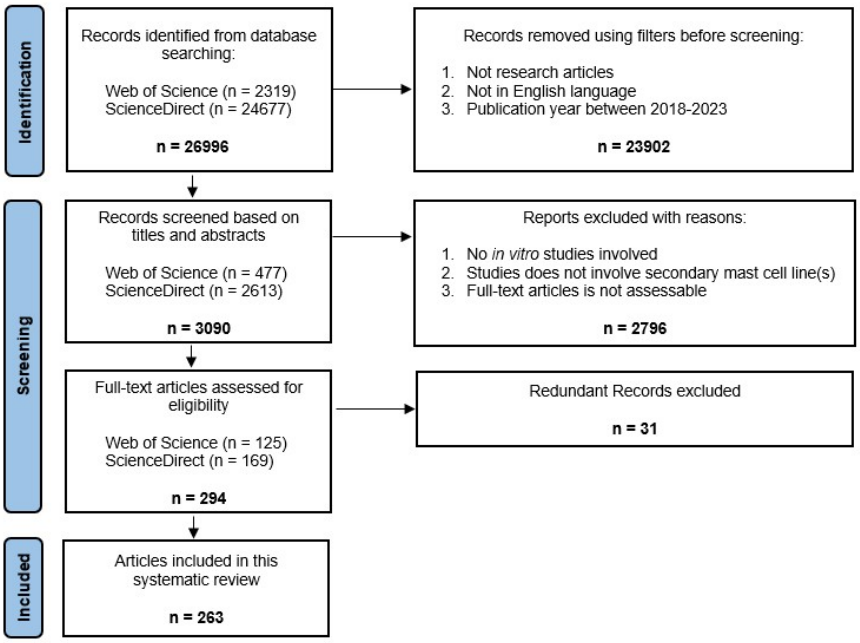
Basal media		**Supplements**			
		Serum		Others	
Name	#articles	Name	#articles	Name	#articles
DMEM	69	10% FBS	91	Penicillin/streptomycin	102
MEM	44	15% FBS	26	L-glutamine/ GlutaMAX	16
α-MEM	2	20% FBS	1	Sodium pyruvate	14
RPMI	3	5% FBS	1	NEAA	7
EMEM	1	17% FBS	1		
Mix α-MEM and RPMI	1				
Mix MEM and IMDM	1				

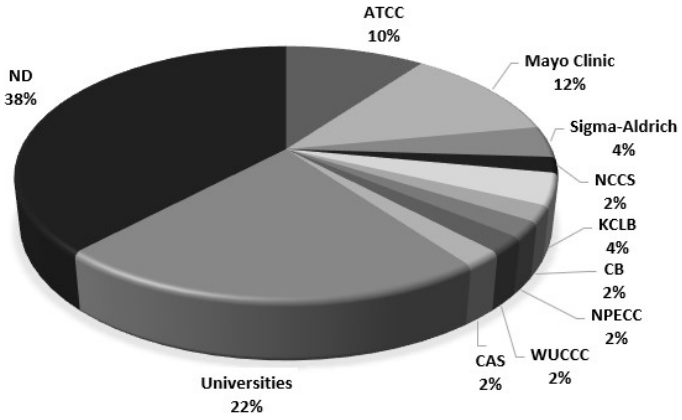
DMEM: Dulbecco's Modified Eagle Medium; MEM: Minimum Essential Media; α-MEM: Minimum Essential Media Eagle–alpha modification; EMEM: Eagle's Minimal Essential Medium; RPMI-1640: Roswell Park Memorial Institute 1640; IMDM: Iscove's Modified Dulbecco's Medium; FBS: fetal bovine serum; NEAA: non-essential amino acids

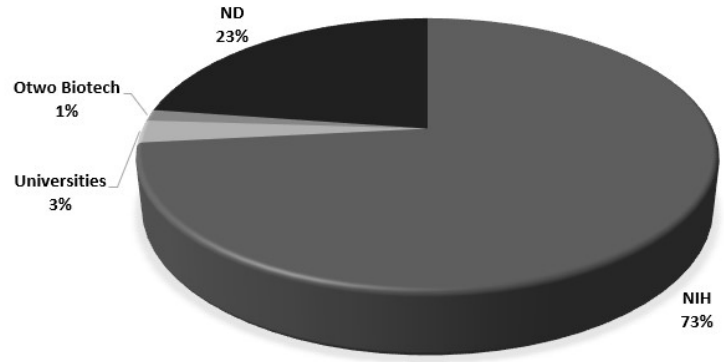
Table 6. Detailed information on the number of articles for the type of inducers, their induction time, and the type of mediators studied in RBL-2H3 cells activation.

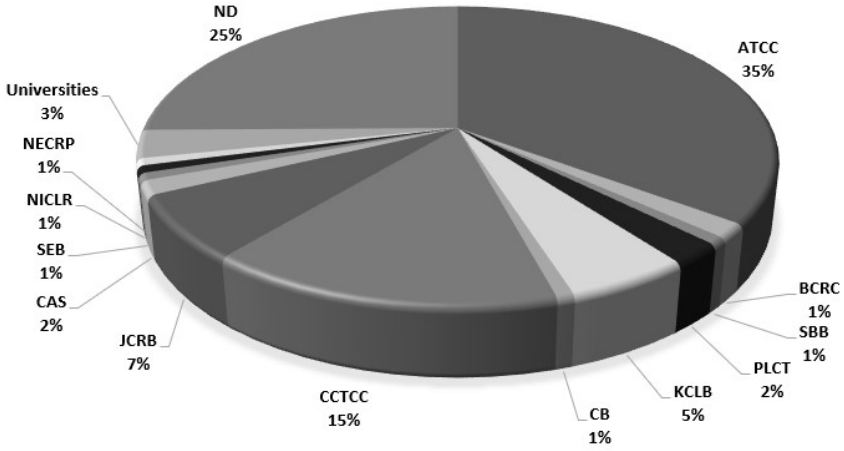
Types of inducers		**Induction**		**Type of mediators**	
Name	# articles	Time	# articles	Name	# articles
DNP-HSA	91	<1h	91	β-hexosaminidase	115
A23187	10	2h-4h	5	Histamine	61
C48/80	15	5h-8h	18	TNF-α	41
DNP-BSA	9	9h-12h	2	IL-1β	6
Substance P	3	13h-24h	19	IL-2	1
PMA	4	Unknown	10	IL-3	2
Patient Sera	2			IL-4	40
TNP-HSA	1			IL-5	4
TNP-BSA	1			IL-6	153
TNP-OVA	1			IL-8	6
Shrimp TM	1			IL-10	4
Oyster TM (Cra g 1)	1			IL-13	8
HDM	1			IL-18	2
Sodium Sulfite	1			IFN-γ	5
PM2.5 suspension	1			Prostaglandin D2	4
Sodium metabisulphite	1			Prostaglandin E2	1
Unknown	1			MCP-1	2
				VEGF	1
				sICAM-1	1
				COX-2	1
				LTC4	4
				5-HT	1
				CCL-2	2
				CCL-17	1
				CCL-22	1
				CCL-25	1
				Tryptase	1

DNP: 2,4-dinitrophenyl; BSA: bovine serum albumin; HSA: human serum albumin; PMA: phorbol-12-myristate-13-acetate; TNP: trinitrophenyl; TM: tropomyosin; HDM: house dust mite; PM: particulate matter; TNF: tumor necrosis factor; IL: interleukin; IFN: interferon; MCP: monocyte chemoattractant protein; VEGF: vascular endothelial growth factor; sICAM: soluble intercellular adhesion molecule; COX: cyclooxygenase; LTC: leukotriene; 5-HT: 5-hydroxytryptamine; CCL: CC motif chemokine ligand

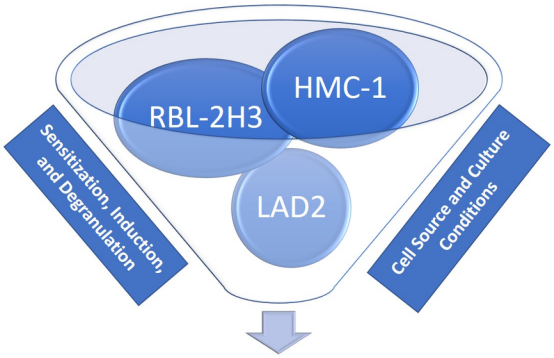








Common *In Vitro* Mast Cell Lines Used in Allergy Studies



Cellular and Molecular Mechanism of Allergy