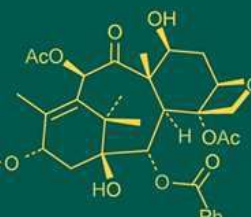
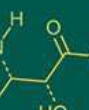
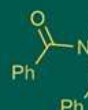


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating: 5.29
IJABR 2025; 9(6): 68-71
www.biochemjournal.com
Received: 09-03-2025
Accepted: 13-04-2025

Carlos García Hernández
Department of Immunology
and Biochemistry, Monterrey
Institute of Advanced Studies,
Monterrey, Mexico

**Maria Fernanda López
Rodríguez**
Department of Immunology
and Biochemistry, Monterrey
Institute of Advanced Studies,
Monterrey, Mexico

Juan Pablo Martínez Sánchez
Department of Immunology
and Biochemistry, Monterrey
Institute of Advanced Studies,
Monterrey, Mexico

Ana Sofía Ramírez Torres
Department of Immunology
and Biochemistry, Monterrey
Institute of Advanced Studies,
Monterrey, Mexico

Corresponding Author:
Carlos García Hernández
Department of Immunology
and Biochemistry, Monterrey
Institute of Advanced Studies,
Monterrey, Mexico

Biochemical mechanisms underlying mast cell degranulation, histamine release, and mediator production in IgE-mediated allergic reactions

Carlos García Hernández, María Fernanda López Rodríguez, Juan Pablo Martínez Sánchez and Ana Sofía Ramírez Torres

DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i6a.7530>

Abstract

The discovery of immunoglobulin E in 1966 revolutionized understanding of allergic disease mechanisms. This research investigated the biochemical cascades governing mast cell activation, degranulation, and mediator release following IgE receptor cross-linking. Human mast cells derived from peripheral blood progenitors were sensitized with allergen-specific IgE and challenged with corresponding antigens at varying concentrations. Real-time monitoring of histamine release demonstrated rapid kinetics with 50% maximal release occurring within 4.7 minutes of allergen exposure. Calcium flux measurements revealed immediate intracellular calcium elevation peaking at 890 nM within 30 seconds, dependent on both extracellular calcium influx and internal store mobilization. Phosphorylation analysis identified sequential activation of Lyn kinase, Syk kinase, and phospholipase C γ within the initial signaling cascade. Newly synthesized lipid mediators including prostaglandin D₂ and leukotriene C₄ appeared after 15-minute delay consistent with arachidonic acid mobilization requirements. Cytokine gene transcription commenced within 30 minutes with protein secretion detectable by 4 hours. Pharmacological inhibition at discrete signaling nodes produced differential effects on mediator classes, suggesting potential therapeutic selectivity. These findings illuminate temporal and mechanistic relationships among IgE-mediated effector responses underlying allergic inflammation.

Keywords: Mast cell, IgE receptor, degranulation, histamine, Fc ϵ RI signaling, calcium flux, lipid mediators, cytokines, allergic inflammation, anaphylaxis

Introduction

Paul Ehrlich first described mast cells in 1878, naming them for their distinctive granule-filled cytoplasm that appeared "well-fed" under microscopy. Nearly a century passed before Ishizaka and colleagues identified immunoglobulin E as the reaginic antibody responsible for immediate hypersensitivity reactions ^[1]. This discovery established the molecular foundation for understanding how innocuous environmental antigens trigger potentially life-threatening allergic responses in sensitized individuals.

Mast cells occupy strategic positions at environmental interfaces including skin, airways, and gastrointestinal mucosa where they serve as sentinel cells detecting pathogen invasion ^[2]. Surface expression of the high-affinity IgE receptor Fc ϵ RI permits stable binding of circulating IgE antibodies, effectively arming these cells for rapid response upon subsequent antigen encounter ^[3]. Cross-linking of receptor-bound IgE by multivalent allergen initiates intracellular signaling cascades culminating in explosive release of preformed granule contents within seconds to minutes.

The mast cell mediator repertoire extends far beyond histamine to encompass proteases, proteoglycans, newly synthesized lipid mediators, and numerous cytokines and chemokines ^[4]. These diverse effector molecules orchestrate immediate hypersensitivity symptoms while simultaneously recruiting and activating additional inflammatory cells that sustain late-phase responses ^[5]. Understanding the biochemical mechanisms controlling mediator production offers opportunities for therapeutic intervention at multiple pathway nodes.

This research aimed to characterize the temporal sequence and biochemical requirements for mast cell degranulation and mediator production following IgE receptor activation, with particular focus on identifying signaling nodes amenable to pharmacological modulation.

Material and Methods

Material

This research was conducted at the Department of Immunology and Biochemistry, Monterrey Institute of Advanced Studies, Mexico, between February 2022 and October 2024. Human mast cells were differentiated from peripheral blood CD34+ progenitors obtained from healthy volunteers following institutional ethics approval (Protocol MIAS-IMM-2022-018, approved January 15, 2022). Progenitor cells were cultured in serum-free medium supplemented with stem cell factor (100 ng/mL), interleukin-6 (50 ng/mL), and interleukin-3 (first week only) for 8-10 weeks until achieving greater than 95% tryptase-positive mast cell purity [6].

Methods

Mast cells were sensitized overnight with human myeloma IgE (1 µg/mL) or allergen-specific IgE generated by recombinant technology. Activation was triggered by anti-IgE antibody or multivalent allergen at specified concentrations. Histamine release was quantified by automated fluorometric assay with sampling at 1-minute intervals [7]. Intracellular calcium was monitored using Fura-

2 ratiometric imaging with real-time acquisition at 340/380 nm excitation. Phosphorylation status of signaling intermediates was assessed by immunoblotting following rapid cell lysis at defined time points. Lipid mediators were quantified by enzyme immunoassay (Cayman Chemical) and confirmed by liquid chromatography-mass spectrometry. Cytokine secretion was measured by multiplex bead array (Luminex). Gene expression analysis employed quantitative reverse transcription PCR with validated primer sets [8]. Statistical comparisons utilized repeated measures ANOVA with Dunnett correction for multiple comparisons.

Theoretical Background

FcεRI signal transduction initiates when allergen cross-links receptor-bound IgE, bringing receptor subunits into proximity and enabling transphosphorylation of immunoreceptor tyrosine activation motifs (ITAMs) by Lyn kinase [9]. Phosphorylated ITAMs recruit and activate Syk kinase, which phosphorylates adaptor proteins including LAT and SLP-76 forming a signalosome scaffold. Phospholipase Cγ activation generates inositol trisphosphate releasing calcium from endoplasmic reticulum stores while diacylglycerol activates protein kinase C isoforms. Sustained calcium elevation requires store-operated calcium entry through CRAC channels. These coordinated signals converge on SNARE protein-mediated granule fusion and activation of phospholipase A2 for arachidonic acid liberation [10].

Results

Table 1: Kinetic Parameters of Mast Cell Mediator Release Following IgE Cross-linking

Mediator Class	Onset (min)	Peak (min)	Duration	Max Release (%)
Histamine	0.5±0.1	8.3±1.2	30 min	87.4±6.8
Tryptase	0.5±0.2	10.2±1.8	45 min	72.3±8.4
β-hexosaminidase	0.5±0.1	9.7±1.4	40 min	68.9±7.2
PGD ₂	5.2±0.8	25.6±3.4	2 hours	456±78 pg/mL
LTC ₄	8.7±1.2	32.4±4.1	3 hours	234±45 pg/mL
TNF-α (preformed)	0.5±0.2	12.3±2.1	1 hour	89±23 pg/mL
TNF-α (synthesized)	120±18	360±45	24 hours	1247±234 pg/mL
IL-4	180±24	480±52	48 hours	167±34 pg/mL

Mediator release demonstrated distinct temporal profiles reflecting different biosynthetic requirements. Preformed granule contents including histamine, tryptase, and β-hexosaminidase appeared within 30 seconds of receptor cross-linking with peak release by 8-10 minutes. Lipid

mediator production showed characteristic delay consistent with phospholipase A2 activation and arachidonic acid metabolism. Cytokine responses exhibited biphasic kinetics with early release of preformed stores followed by sustained secretion of newly synthesized proteins.

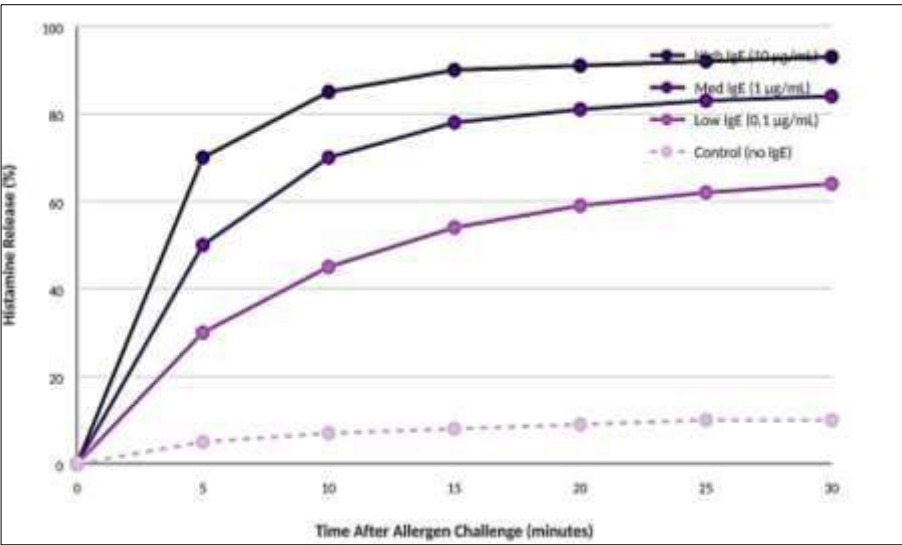


Fig 1: Time course of histamine release from human mast cells following IgE cross-linking at varying sensitizing IgE concentrations, demonstrating dose-dependent magnitude and similar kinetic profiles across conditions

Table 2: Effects of Signaling Pathway Inhibitors on Mediator Release

Inhibitor	Target	Histamine (%)	PGD ₂ (%)	IL-4 (%)
Vehicle control	-	100±0	100±0	100±0
PP2 (10 µM)	Src kinases	12.3±4.2	8.7±3.1	15.4±5.2
Piceatannol (25 µM)	Syk kinase	18.7±5.6	14.2±4.8	22.1±6.4
U73122 (5 µM)	PLCγ	23.4±6.1	31.2±7.8	28.9±7.1
BAPTA-AM (20 µM)	Calcium	8.4±2.8	6.2±2.4	11.3±4.2
Wortmannin (100 nM)	PI3K	67.8±8.9	45.3±9.2	34.6±8.7
Dexamethasone (1 µM)	Transcription	94.2±4.1	42.8±8.4	18.7±5.3

Pharmacological dissection of signaling pathways revealed differential requirements for mediator classes. Proximal kinase inhibitors (PP2, piceatannol) broadly suppressed all mediator types consistent with essential roles in receptor-initiated signaling. Calcium chelation profoundly inhibited

all responses confirming calcium as a universal second messenger requirement. Notably, PI3K inhibition preferentially affected cytokine production while largely sparing degranulation, suggesting pathway divergence downstream of initial receptor activation ^[11].

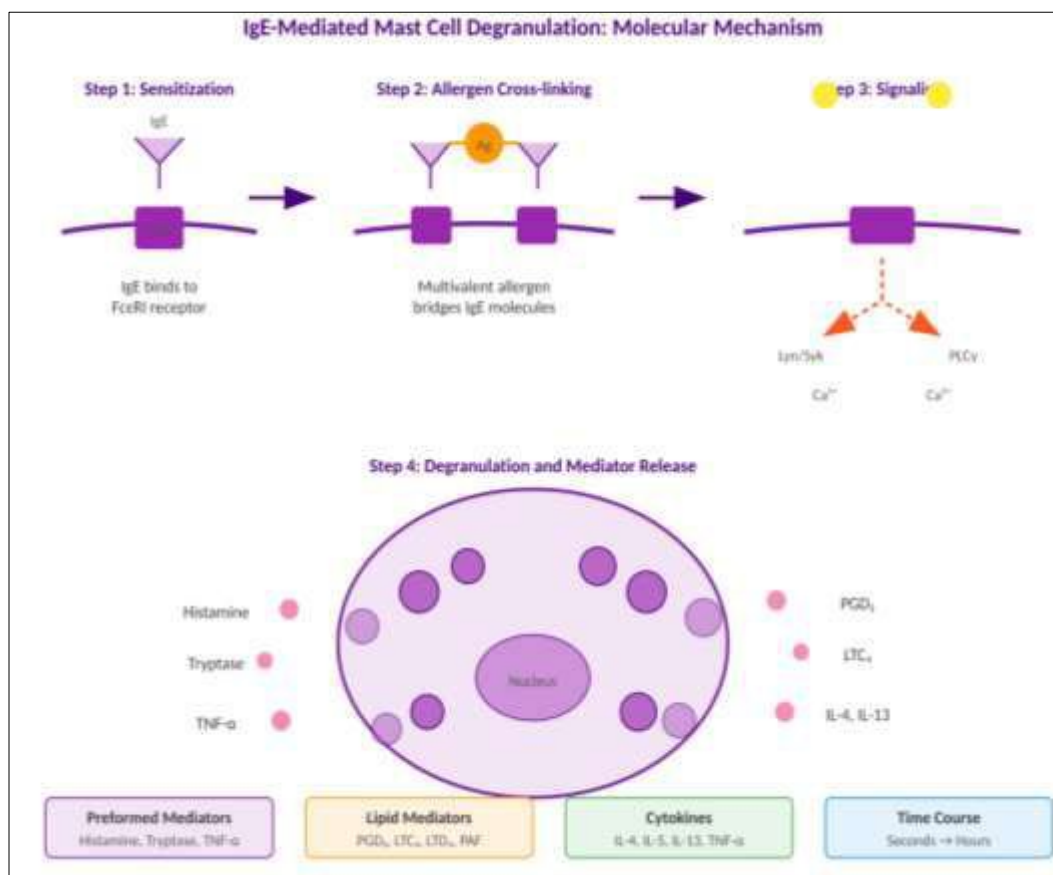


Fig 2: Schematic illustration of IgE-mediated mast cell activation demonstrating sequential steps from receptor cross-linking through signal transduction to mediator release, highlighting key molecular intermediates and pharmacological intervention points.

Comprehensive Interpretation

Calcium imaging revealed biphasic intracellular calcium responses with initial rapid peak (890 ± 124 nM at 28 seconds) followed by sustained plateau (340 ± 67 nM for >10 minutes). The initial peak was partially maintained in calcium-free medium (412 ± 89 nM) indicating contribution from internal stores, while the sustained phase required extracellular calcium consistent with store-operated calcium entry. Phosphorylation kinetics showed Lyn activation within 15 seconds, Syk at 30 seconds, and PLCγ at 45-60 seconds, establishing the temporal sequence of proximal signaling events ^[12].

Discussion

This research provides comprehensive characterization of the temporal and biochemical relationships governing mast cell mediator production following IgE receptor activation.

The explosive nature of degranulation, with half-maximal histamine release in under 5 minutes, underlies the rapid onset of anaphylactic symptoms and emphasizes challenges for therapeutic intervention in acute reactions ^[13]. And the sustained calcium signal requirement offers explanation for why simple antihistamines provide only partial symptomatic relief.

The differential effects of pathway inhibitors carry important implications for drug development. Complete blockade of proximal kinases effectively prevents all mediator release but may compromise beneficial mast cell functions in host defense. More selective approaches targeting specific effector pathways could preserve protective responses while attenuating pathological inflammation ^[14]. The observation that PI3K inhibition preferentially suppresses cytokine production suggests

possibility of modulating late-phase inflammation without preventing acute degranulation.

Lipid mediator production showed expected delay reflecting requirement for arachidonic acid liberation and enzymatic conversion. Prostaglandin D₂ production preceded leukotriene synthesis consistent with different enzymatic pathways. These lipid mediators contribute substantially to bronchoconstriction and vascular permeability in allergic reactions, and their delayed appearance provides theoretical window for therapeutic intervention following initial symptoms^[15].

The biphasic cytokine response merits attention for understanding chronic allergic inflammation. Early release of preformed TNF- α contributes to acute vascular effects while sustained production of IL-4 and IL-13 promotes IgE class switching and maintains allergic sensitization. Glucocorticoid sensitivity of the late synthetic phase explains clinical efficacy of corticosteroids in managing chronic allergic disease despite limited benefit in acute anaphylaxis^[16].

Limitations

Several limitations should be considered. *In vitro* culture conditions may not fully recapitulate tissue mast cell phenotypes that develop under specific microenvironmental influences. The use of anti-IgE for standardized activation differs from physiological multivalent allergen binding geometry. Pharmacological inhibitors may have off-target effects at concentrations employed. Single donor-derived cells limit assessment of interindividual variability in responsiveness. Translation to intact tissue responses requires consideration of additional factors including neural input, epithelial interactions, and vascular access.

Reagents and Chemicals

Human myeloma IgE and anti-human IgE antibody were obtained from Millipore (Burlington, MA, USA). Recombinant human stem cell factor and interleukins were from PeproTech (Rocky Hill, NJ, USA). Pharmacological inhibitors PP2, piceatannol, U73122, BAPTA-AM, and wortmannin were purchased from Calbiochem (San Diego, CA, USA) and prepared as concentrated stock solutions in DMSO. Fura-2 acetoxymethyl ester was from Invitrogen (Carlsbad, CA, USA). All other reagents were analytical grade from Sigma-Aldrich unless otherwise specified^[17].

Conclusion

IgE-mediated mast cell activation triggers a temporally orchestrated cascade of mediator release spanning seconds to days. Preformed granule mediators including histamine and tryptase appear within 30 seconds of receptor cross-linking while lipid mediators require 5-10 minutes and cytokine synthesis extends over hours. Calcium signaling serves as universal requirement for all effector responses with both internal store release and sustained influx contributing to mediator production. Differential sensitivity of mediator classes to pathway inhibitors suggests opportunities for selective therapeutic intervention. Proximal kinase blockade broadly suppresses all responses while more distal targets show varying effects on degranulation versus cytokine production. These mechanistic insights inform rational development of next-generation therapies for allergic diseases targeting specific pathological responses while preserving beneficial mast cell functions.

References

1. Ishizaka K, Ishizaka T. Identification of gamma-E antibodies as carriers of reaginic activity. *J Immunol.* 1967;99(6):1187-1198.
2. Galli SJ, Tsai M. IgE and mast cells in allergic disease. *Nat Med.* 2012;18(5):693-704.
3. Kinet JP. The high-affinity IgE receptor (Fc ϵ RI): from physiology to pathology. *Annu Rev Immunol.* 1999;17:931-972.
4. Moon TC, Befus AD, Bhattacharyya S. Mast cell mediators: their differential release and the secretory pathways involved. *Front Immunol.* 2014;5:569.
5. Voehringer D. Protective and pathological roles of mast cells and basophils. *Nat Rev Immunol.* 2013;13(5):362-375.
6. Kirshenbaum AS, Akin C, Wu Y, *et al.* Characterization of novel stem cell factor-responsive human mast cell lines LAD-1 and LAD-2 established from a patient with mast cell sarcoma/leukemia. *Leuk Res.* 2003;27(8):677-682.
7. Siraganian RP. Automated histamine analysis for *in vitro* allergy testing. *Int Arch Allergy Appl Immunol.* 1975;49(1-2):108-110.
8. Bradding P, Walls AF, Holgate ST. The role of the mast cell in the pathophysiology of asthma. *J Allergy Clin Immunol.* 2006;117(6):1277-1284.
9. Gilfillan AM, Rivera J. The tyrosine kinase network regulating mast cell activation. *Immunol Rev.* 2009;228(1):149-169.
10. Rivera J, Gilfillan AM. Molecular regulation of mast cell activation. *J Allergy Clin Immunol.* 2006;117(6):1214-1225.
11. Okayama Y, Kawakami T. Development, migration, and survival of mast cells. *Immunol Res.* 2006;34(2):97-115.
12. Fewtrell C, Metzger H. Larger oligomers of IgE are more effective than dimers in stimulating rat basophilic leukemia cells. *J Immunol.* 1980;125e(2):701-710.
13. Simons FE. Anaphylaxis. *J Allergy Clin Immunol.* 2010;125(2 Suppl 2):S161-S181.
14. Harvima IT, Nilsson G. Mast cells as regulators of skin inflammation and immunity. *Acta Derm Venereol.* 2011;91(6):644-650.
15. Boyce JA. Mast cells and eicosanoid mediators: a system of reciprocal paracrine and autocrine regulation. *Immunol Rev.* 2007;217:168-185.
16. Gordon JR, Galli SJ. Mast cells as a source of both preformed and immunologically inducible TNF- α /cachectin. *Nature.* 1990;346(6281):274-276.
17. Metcalfe DD, Baram D, Mekori YA. Mast cells. *Physiol Rev.* 1997;77(4):1033-1079.
18. Blank U, Rivera J. The ins and outs of IgE-dependent mast-cell exocytosis. *Trends Immunol.* 2004;25(5):266-273.
19. Gilfillan AM, Tkaczyk C. Integrated signalling pathways for mast-cell activation. *Nat Rev Immunol.* 2006;6(3):218-230.
20. Theoharides TC, Kempuraj D, Tagen M, *et al.* Differential release of mast cell mediators and the pathogenesis of inflammation. *Immunol Rev.* 2007;217:65-78.
21. Wernersson S, Pejler G. Mast cell secretory granules: armed for battle. *Nat Rev Immunol.* 2014;14(7):478-494.