

Analysis of the Mechanism for the Development of Allergic Skin Inflammation and the Application for Its Treatment: Aspirin Modulation of IgE-Dependent Mast Cell Activation: Role of Aspirin-Induced Exacerbation of Immediate Allergy

Yoshihiro Suzuki^{1,*} and Chisei Ra¹

¹Division of Molecular Cell Immunology and Allergology, Nihon University Graduate School of Medical Science, Tokyo 173-8610, Japan

Received December 5, 2008; Accepted March 6, 2009

Abstract. Aspirin (acetylsalicylic acid) is a well-known nonsteroidal anti-inflammatory drug that can potentiate some acute allergies and causes adverse immunological reactions collectively referred to as aspirin intolerance, a disorder that induces urticaria, asthma, and anaphylaxis in response to oral administration of the drug. Aspirin also potentiates some acute allergies such as food-dependent exercise-induced anaphylaxis (FDEIA), a food allergy induced by physical exercise. The anti-inflammatory actions as well as the adverse immunological effects have been thought to be primarily due to inhibition of cyclooxygenase activity. However, a growing body of evidence suggests that mechanisms unrelated to inhibition of prostaglandin synthesis are involved. One key feature of aspirin intolerance is the overproductions of cysteinyl leukotrienes (LTs), in which mast cells have been implicated to play a role. In this review, we provide an overview of our current knowledge about the regulatory mechanisms of LTC₄ secretion in mast cells and its modulation by aspirin, with a special emphasis on the role of Ca²⁺ signals. We also introduced our recent findings that mast cells express dihydropyridine-sensitive L-type Ca²⁺ channels (LTCCs) and that Ca²⁺ channels of this kind mediate aspirin modulation of LTC₄ secretion in mast cells.

Keywords: mast cell, aspirin, L-type Ca²⁺ channel, leukotriene, allergic inflammation

Introduction

Aspirin (acetylsalicylic acid, ASA) is a well-known nonsteroidal anti-inflammatory drug (NSAID) that can potentiate some acute allergies and causes adverse immunological reactions collectively referred to as aspirin intolerance. ASA intolerance is a disorder that induces urticaria, asthma, and anaphylaxis in response to oral administration of the drug (1, 2). ASA also potentiates some acute allergies such as food-dependent exercise-induced anaphylaxis (FDEIA), a food allergy induced by physical exercise. Recently, ASA was shown to act as a powerful trigger of anaphylaxis in FDEIA patients (3). The anti-inflammatory actions of ASA have

been attributed primarily to inhibition of prostaglandin synthesis (4). ASA acetylates Ser-530 of cyclooxygenase (COX) I and II, thereby blocking prostaglandin and thromboxane A₂ synthesis, while therapeutic concentrations of ASA and salicylates (SA) inhibit COX II protein expression (5). An emerging view is that additional mechanisms, independent of COX inhibition, are involved in the anti-inflammatory actions of ASA and SA. These mechanisms include inhibiting the activity of the transcription factors nuclear factor κ B (NF- κ B) (6) and nuclear factor of activated T cells (NF-AT) (7), as well as inhibiting degranulation of basophils and mast cells (8).

One key feature of ASA intolerance is the overproduction of cysteinyl leukotrienes (cys-LTs) such as LTC₄/D₄/E₄, which are all sequentially synthesized from arachidonic acid (AA). The cys-LTs are potent proinflammatory mediators and cause smooth muscle contraction and increased vascular permeability. Patients

*Corresponding author. ysuzuki@med.nihon-u.ac.jp
Published online in J-STAGE
doi: 10.1254/jphs.08R32FM

with ASA intolerance have significantly higher levels of cys-LTs in their bronchoalveolar lavage fluid and urine before and after oral ASA challenge (9).

Mast cells are important effector cells in allergic and inflammatory reactions and are the major producers of cys-LTs. Mast cells express the high-affinity IgE receptor (Fc ϵ RI) on their cell surface, and antigen cross-linking of Fc ϵ RI triggers biochemical cascades that lead to degranulation, cys-LTs and PGs secretion, and cytokine production. Moreover, mast cells are suggested to be responsible for cys-LT overproduction in ASA intolerance (10, 11). In addition, several studies have shown that an atopic background (high levels of serum IgE) is a risk factor for NSAID sensitivity (12).

Dual effects of ASA and SA on IgE + Ag-mediated LTC₄ secretion in mast cells

We analyzed the effects of ASA on IgE + antigen (Ag)-mediated LTC₄ secretion and on the signaling pathways involved in secretion in cultured mast cells including RBL-2H3 cells and murine bone marrow-derived mast cells (BMMCs) (13). Cells that had been passively sensitized with anti-TNP IgE were treated with ASA and then immediately stimulated with the Ag TNP-BSA (the basal LTC₄ level, 48 ± 11 pg/ml; IgE + Ag stimulation, 3601 ± 218 pg/ml, $n = 5$). Mortaz et al. (8) have reported that 10 mM ASA alone increases LT release, whereas ASA inhibits IgE + Ag-mediated β -hexosaminidase release. In both cell types, however, 10 mM ASA exhibited substantial cytotoxicity, and ASA up to 3 mM had a no effect on LT release. ASA

had a dual effect on IgE + Ag-mediated LTC₄ secretion, depending on the concentration employed: at low concentrations (≤ 0.3 mM), ASA significantly enhanced LTC₄ secretion, while at high concentrations (>1 mM), it suppressed secretion (Fig. 1A). The optimal concentration of ASA was 0.1–0.3 mM in different experiments (approximately 1.5-fold greater secretion compared to IgE + Ag stimulation alone). Similar results were obtained when cells were exposed to the aspirin metabolite SA: at low concentrations (≤ 0.3 mM), SA significantly enhanced IgE + Ag-mediated LTC₄ secretion, while at high concentrations (>1 mM), it suppressed secretion (Fig. 1B). Essentially similar results were obtained with BMMCs (13). SA was approximately 3-fold more toxic than ASA. Treatment with ASA up to 3 mM for 4 h had a minimal effect on cell viability, as measured by phosphatidylserine externalization and propidium iodide permeability or trypan blue dye exclusion assay (data not shown). DMSO in 3 mM ASA (0.3%) had minimal effects on secretion and viability (97%). These results demonstrate that ASA and SA exhibit stimulatory effects on IgE + Ag-mediated LTC₄ secretion at non-toxic concentrations. Since ASA suppresses COX-1 and COX-2 activities and SA does not (14), these results suggest that the enhancement of LTC₄ secretion is not via suppression of COX activity.

ASA enhances cPLA₂ activation independently of the ERK and p38MAPK pathways

To gain insight into the mechanisms by which ASA modulates LTC₄ secretion in mast cells, we examined the

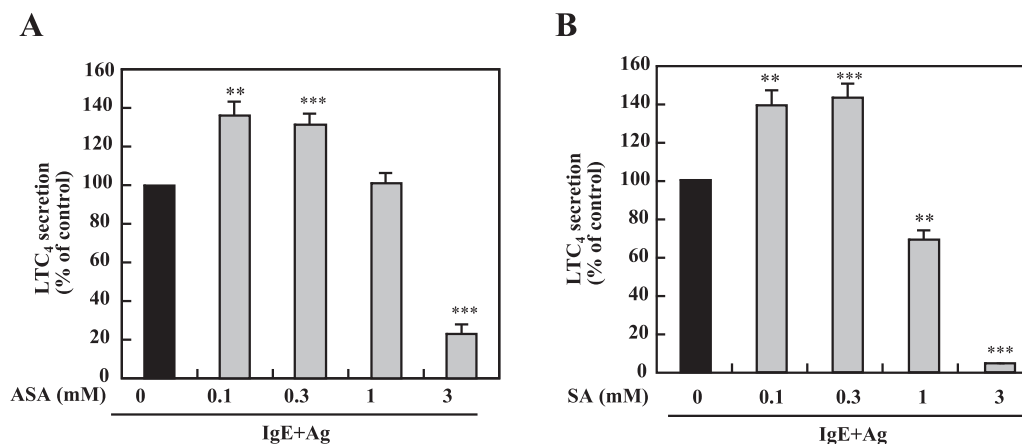


Fig. 1. Effects of ASA and SA on IgE + Ag-mediated LTC₄ secretion in RBL-2H3 mast cells. RBL-2H3 cells that had been sensitized with anti-TNP IgE were treated with ASA or SA at the indicated concentrations and then immediately stimulated with 30 ng/ml TNP-BSA (Ag) for 30 min at 37°C. The LTC₄ content of the supernatant was determined by ELISA. The results are presented as a percent of the control, in which the LTC₄ secretion in cells stimulated with IgE + Ag in the absence of drug is 100%. The data are the mean $\pm$ S.E.M. of three to five independent experiments. ** $P < 0.01$, *** $P < 0.001$. Modified with permission from Clin Immunol (Ref. 13).

effect of ASA on the intracellular signaling pathways involved in LTC₄ synthesis, including activation of cytosolic phospholipase A₂ (cPLA₂), which mediates agonist-induced AA release in most cell types (15). The catalytic activity of cPLA₂ is phosphorylation-dependent. Phosphorylation of cPLA₂ on Ser-505 by ERK1/2 is necessary for cPLA₂-mediated AA release following stimulation of various cell types by many different agonists (16, 17). By using a monoclonal antibody that specifically recognizes the Ser-505 phosphorylation of cPLA₂, we found that IgE + Ag stimulation substantially induced cPLA₂ phosphorylation, and that low concentrations (≤ 0.3 mM) of ASA substantially (1.5-fold) enhanced the effect (Fig. 2A). Thus, ASA enhanced both of IgE + Ag-mediated LTC₄ secretion and Ser-505 phosphorylation of cPLA₂. We next examined whether ASA stimulated activation of ERK1/2. ERK1/2 is activated following dual phosphorylation of a threonine and a tyrosine residue by the upstream kinase MEK1/2. RBL-2H3 cells were treated with various concentrations (0.1–3 mM) of ASA prior to IgE + Ag stimulation, and activation of ERK1/2 was then assessed using a monoclonal antibody that specifically recognizes the dually phosphorylated (active) ERK1/2 (Thr²⁰²/Tyr²⁰⁴) (18). The phosphorylation of p42/44 ERK1/2 clearly increased following IgE + Ag stimulation (Fig. 2B). However, ASA treatment dose-dependently reduced the activation of ERK1/2 (Fig. 2B). The cPLA₂ Ser-727 residue is another site important for activation of the enzyme and is phosphorylated by MNK1-related protein kinases, which are activated by p38MAPK (19). We therefore examined the effect of ASA on p38MAPK activation. Western blot analysis using a monoclonal antibody recognizing the active enzyme, phospho-p38MAPK (Thr180/Tyr182), showed that IgE + Ag stimulation induced p38MAPK activation, but ASA at concentrations ranging from 0.1 to 3 mM did not substantially affect the phosphorylation level (Fig. 2C). To determine the involvement of these MAPKs in LTC₄ secretion in our cell system, the effect of pharmacological inhibitors of selective MAPK pathways on IgE + Ag-mediated LTC₄ secretion was examined. U0126 (20 μ M) and SB203580 (30 μ M), which are selective inhibitors of ERK1/2 and p38MAPK, respectively, substantially (up to 50% inhibition) inhibited IgE + Ag-mediated LTC₄ secretion in RBL-2H3 cells, indicating that both MAPK pathways are involved in LTC₄ synthesis in our cell system. Collectively, our data indicate that ASA enhances cPLA₂ activation independently of the ERK and p38MAPK pathways.

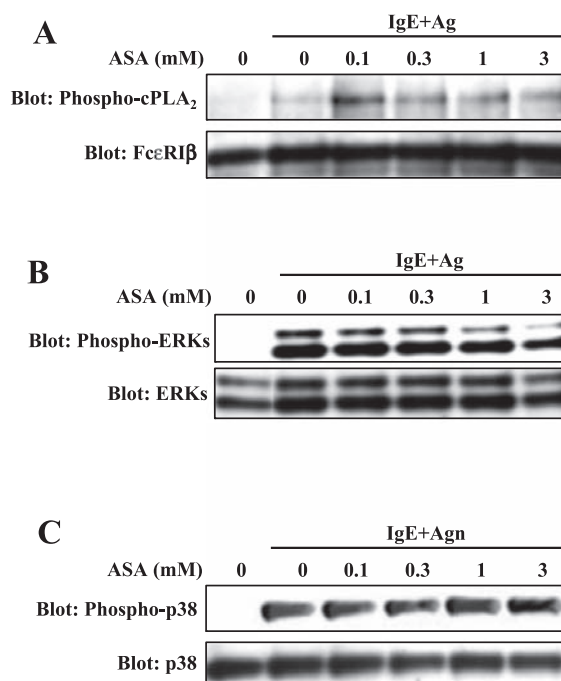


Fig. 2. ASA stimulates activation of cPLA₂ independently of ERK1/2 and p38MAPK pathways. A–C: RBL-2H3 cells were treated with ASA at the indicated concentrations and then immediately stimulated with 30 ng/ml Ag for 5 min at 37°C. The stimulated cells were lysed with SDS sample buffer. The proteins were separated by SDS-PAGE using a 10% separating gel under reducing conditions and then transferred to PVDF membranes. The presence of phospho-cPLA₂ (A), phospho-p44/42 ERK1/2 (B), and phospho-p38MAPK (C) was analyzed by Western blotting using specific antibodies. To verify equal loading, the blots were reprobed with antibodies to FcεRIβ (A) or each kinase (B, C). The data are representative of three independent experiments. Modified with permission from Clin Immunol (Ref. 13).

IgE + Ag-mediated LTC₄ secretion is Ca²⁺-dependent and ASA has a dual effect on Ca²⁺ responses

Another key physiological regulator of LTC₄ synthesis is Ca²⁺. cPLA₂ is regulated by an increase in intracellular Ca²⁺, which binds to the amino-terminal C2 domain and induces cPLA₂ translocation to the nuclear envelope and the endoplasmic reticulum (20). Ca²⁺ is an important regulator of 5-lipoxygenase (5-LOX) as well, which catalyzes the addition of molecular oxygen to AA. Because Ca²⁺ entry from the extracellular space is a major source of increased [Ca²⁺]_i, we determined whether LTC₄ secretion in mast cells was dependent on Ca²⁺ influx. As shown in Fig. 3A, EGTA chelation of extracellular Ca²⁺ completely abolished IgE-mediated LTC₄ secretion, indicating that this response is strictly dependent on Ca²⁺ influx. We then examined the effect of ASA on Ca²⁺ mobilization. ASA alone at concentrations ranging from 0.1 to 3 mM caused a minimal

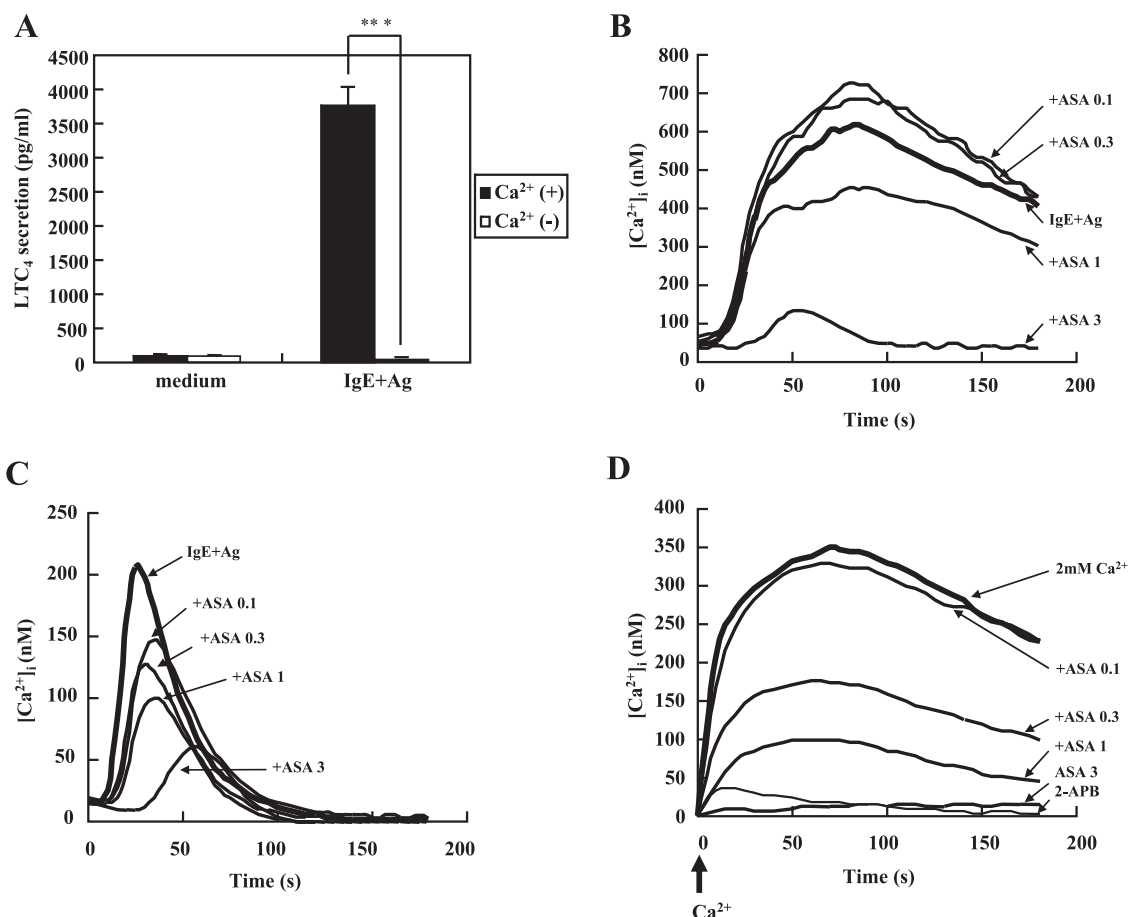


Fig. 3. ASA has a dual effect on IgE + Ag-mediated Ca²⁺ responses. **A:** RBL-2H3 cells were suspended in Ca²⁺-containing medium (HBSS supplemented with 1 mM CaCl₂) or nominally Ca²⁺-free medium (HBSS supplemented with 1 mM EGTA), stimulated with 30 ng/ml Ag for 30 min at 37°C, and then the LTC₄ content of the supernatant was determined by ELISA. The data represent the mean ± S.E.M. of four independent experiments. ****P* < 0.001. **B and C:** RBL-2H3 cells were loaded with Fluo3/AM, washed twice in HBSS, and resuspended in Ca²⁺-containing medium (HBSS supplemented with 1 mM CaCl₂) (**B**) or nominally Ca²⁺-free medium (HBSS supplemented with 1 mM EGTA) (**C**). The cells were incubated with ASA at the indicated concentrations and then immediately stimulated with 30 ng/ml Ag. The data are expressed as calculated [Ca²⁺]_i and are representative of two independent experiments. **D:** RBL-2H3 cells loaded with Fluo3/AM were resuspended in nominally Ca²⁺-free medium and treated with thapsigargin (Tg) for 10 min at 37°C in order to deplete intracellular Ca²⁺ stores and activate SOCE. The cells were then incubated with ASA at the indicated concentrations or 10 μM 2-APB prior to the addition of 2 mM CaCl₂ (indicated by the arrow). The data are representative of five independent experiments. Modified with permission from Clin Immunol (Ref. 13).

increase in [Ca²⁺]_i (data not shown). However, ASA had a dual effect on IgE + Ag-mediated [Ca²⁺]_i increase: at low concentrations (≤0.3 mM), ASA enhanced the response, while at high concentrations (>1 mM), it suppressed the [Ca²⁺]_i increase (Fig. 3B). Next, the effect of ASA on Ca²⁺ mobilization from intracellular stores was examined in cells placed in nominally Ca²⁺-free medium. As shown in Fig. 3C, ASA dose-dependently reduced the effect of IgE + Ag stimulation. ASA reduced the maximal level of [Ca²⁺]_i with a minimum effective dose of 0.1 mM and caused an increase in the time to reach peak [Ca²⁺]_i levels (for ASA concentrations of 0, 0.3, 1, and 3 mM, the times to peak [Ca²⁺]_i levels were

30, 35, 50, and 60 s, respectively). In nonexcitable cells including mast cells, store-operated Ca²⁺ entry (SOCE) is a main pathway of Ca²⁺ influx (21). In RBL cells, the CRAC channels, the best known store-operated Ca²⁺ channels (SOCs), are activated by a pharmacological emptying of intracellular stores with thapsigargin (Tg) or by IgE + Ag stimulation (22, 23). We therefore examined whether ASA affected Ca²⁺ influx through SOCs. Cells were treated with Tg for 10 min in nominally Ca²⁺-free medium in order to deplete intracellular Ca²⁺ stores and activate SOCs. A substantial [Ca²⁺]_i rise was seen following readmission of external Ca²⁺ to the Tg-treated cells (Fig. 3D). This Ca²⁺ influx was

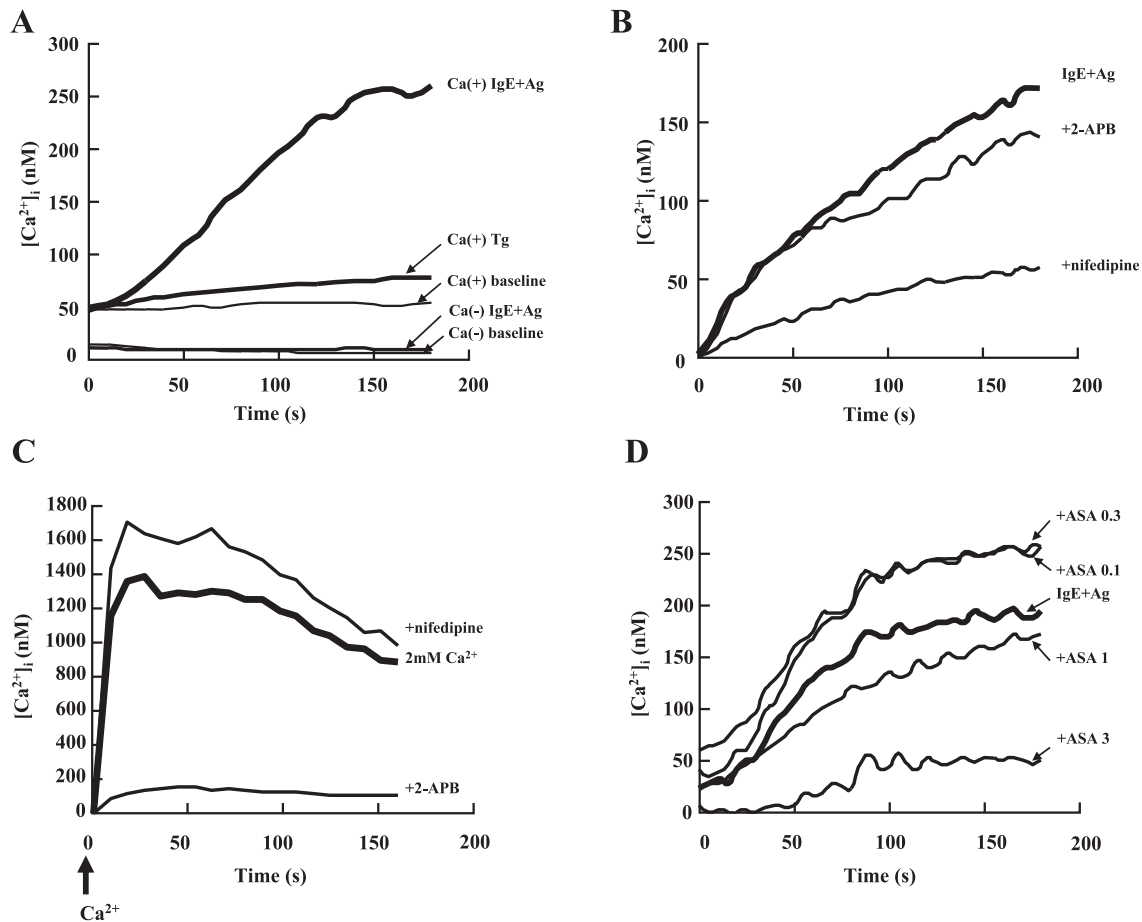


Fig. 4. ASA facilitates Ca^{2+} influx through LTCCs. **A:** RBL-2H3 cells loaded with Fluo3/AM were resuspended in nominally Ca^{2+} -free medium and treated with Tg for 10 min at 37°C. After extensive washing, the store-depleted cells were resuspended in Ca^{2+} -containing medium or nominally Ca^{2+} -free medium and then stimulated with 30 ng/ml Ag or with 1 μ M Tg. **B and D:** The store-depleted cells resuspended in Ca^{2+} -containing medium were incubated with 10 μ M 2-APB or 1 μ M nifedipine (**B**) or with ASA at the indicated concentrations (**D**) and then immediately stimulated with 30 ng/ml Ag. **C:** SOCE was induced in RBL-2H3 cells by pharmacologically depleting intracellular Ca^{2+} stores as described in the legend of Fig. 3D. The cells were treated with 10 μ M 2-APB or 1 μ M nifedipine before Ca^{2+} replenishment (indicated by the arrow). The data are representative of five independent experiments. Modified with permission from Clin Immunol (Ref. 13).

almost completely abolished by 2-APB, a SOCE antagonist, indicating that the influx is SOC mediated. ASA (≥ 0.3 mM) suppressed Ca^{2+} influx in a dose-dependent manner. These results suggest that ASA may stimulate some other Ca^{2+} -entry pathway.

ASA enhances LTC_4 secretion through facilitating LTCCs

Recent work has demonstrated that in various immune cells including T and B cells, in addition to SOCE, Ag-receptor stimulation can activate Ca^{2+} entry independently of Ca^{2+} store emptying. Moreover, we previously reported that IgE + Ag stimulation induces substantial Ca^{2+} entry even when SOCE is inactive. In addition, this Ca^{2+} entry was blocked by LTCC antagonists, but

minimally affected by SOCE antagonists (24), suggesting the occurrence of LTCCs. More recently, we detected the expression of the LTCC α_{1c} ($Ca_v1.2$) transcripts and protein on mast cell surfaces. Inhibition of the LTCC activity by pharmacological antagonists such as nifedipine and diltiazem as well as small interfering RNA knockdown of α_{1c} ($Ca_v1.2$) LTCCs blocks IgE + Ag-mediated interleukin-13 and tumor necrosis factor- α productions, but not degranulation (25). These findings suggest that mast cells express functional LTCCs, which are activated to regulate mediator secretion upon IgE + Ag stimulation. Therefore, we examined whether ASA might target this kind of Ca^{2+} channels. Consistent with our previous studies (24, 25), IgE + Ag stimulation increased $[Ca^{2+}]_i$ in Ca^{2+} store-depleted cells. This effect was absent in Ca^{2+} -free conditions,

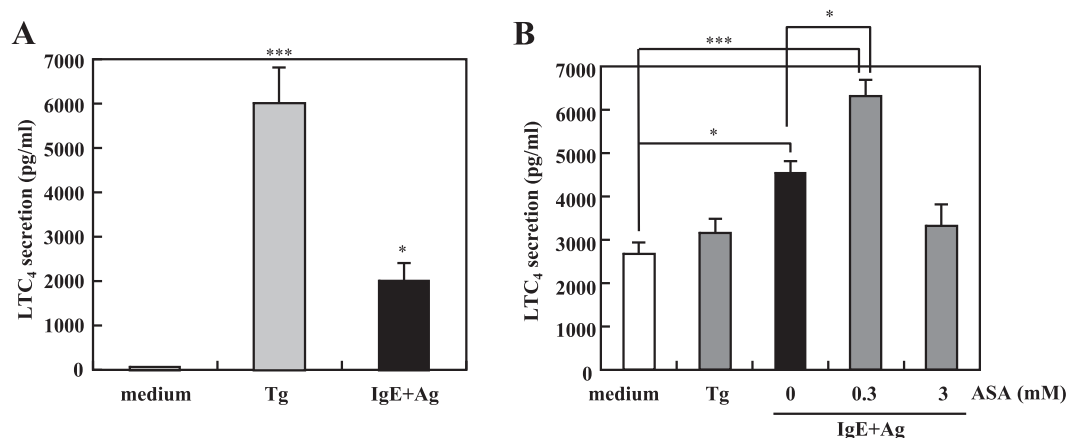


Fig. 5. ASA enhances LTCC-mediated LTC₄ secretion. RBL-2H3 cells were suspended in Ca²⁺-containing medium, stimulated with 30 ng/ml Ag or 1 μ M Tg for 30 min at 37°C, and then the LTC₄ content of the supernatant was determined by ELISA. The data represent the mean $\pm$ S.E.M. of four independent experiments. **P*<0.05, ****P*<0.001. B: RBL-2H3 cells suspended in nominally Ca²⁺-free medium were depleted of intracellular Ca²⁺ stores as described above and then resuspended in Ca²⁺-containing medium. The cells were then stimulated with 30 ng/ml Ag or 1 μ M Tg for 30 min at 37°C in the absence or presence of ASA at the indicated concentrations, and the LTC₄ content of the supernatant was determined by ELISA. The data represent the mean $\pm$ S.E.M. of four independent experiments. **P*<0.05, ****P*<0.001. Modified with permission from Clin Immunol (Ref. 13).

indicating that it was due to entry of extracellular Ca²⁺ (Fig. 4A). This Ca²⁺ entry was substantially reduced by the LTCC antagonist nifedipine but minimally affected by 2-APB. On the other hand, 2-APB completely abolished Ca²⁺ influx through SOCs, while nifedipine enhanced it (Fig. 4: B and C). ASA had a dual effect on this LTCC channel activity: at low concentrations (≤ 0.3 mM), ASA enhanced it, while at high concentrations (>1 mM), suppressed it (Fig. 4D). These results demonstrate that ASA enhances DHP-sensitive LTCCs.

Next, we determined whether the DHP-sensitive LTCCs were involved in LTC₄ secretion. Tg could induce substantial LTC₄ secretion under normal conditions: 1 μ M Tg was more effective than IgE + Ag stimulation with the optimal Ag concentration (Fig. 5A). Depletion of intracellular Ca²⁺ stores by Tg resulted in increased LTC₄ secretion, which may result from activation of SOCE (2689 ± 261 pg/ml, *n* = 6). In accordance with its inability to evoke the Ca²⁺ response, Tg no longer induced any significant increase in LTC₄ secretion in the cells (3142 ± 318 pg/ml, Fig. 5B). In contrast, IgE + Ag stimulation still significantly increased LTC₄ secretion (4520 ± 289 pg/ml), in parallel with substantial Ca²⁺ entry. Moreover, ASA had a dual effect on the LTC₄ secretion: at low concentrations (0.3 mM), ASA significantly enhanced secretion (6311 ± 350 pg/ml, *n* = 4), while a high concentration (3 mM) suppressed secretion (3303 ± 484 pg/ml, *n* = 4, Fig. 5B). Taken together, these findings suggest that ASA modulates IgE + Ag-mediated LTC₄ secretion through affecting the LTCC channel activity.

Concluding remarks

It has been demonstrated that Ca²⁺ influx through CRAC channels regulates ERK activation, thereby controlling cPLA₂ activation and LTC₄ secretion (22, 23). We demonstrated here the occurrence of another Ca²⁺-dependent pathway that leads to cPLA₂ activation and LTC₄ secretion independently of ERK activation. This pathway is mediated by DHP-sensitive LTCCs, which are pharmacologically distinct from SOCs (Fig. 6). Furthermore, it was demonstrated that ASA modulates LTC₄ secretion through this LTCC-mediated pathway. Although the biological significance of these findings remained to be elucidated, it is noteworthy that the LTCC-mediated LTC₄ secretion is enhanced by mitochondrial depolarization, while the SOC-mediated LTC₄ secretion is blocked (13). In the inflammatory milieu, mast cells may be exposed to oxidative stress, the major cause of mitochondrial depolarization, leading to inactivation of the SOC-mediated LTC₄ secretion. However, in the presence of ASA (especially at low doses), mast cells may produce substantially LTC₄ secretion through the LTCC pathway, leading to the exacerbation of IgE-mediated allergic reactions. Better understanding of the molecular mechanisms underlying ASA/ASA modulation of LTCCs could lead to a new approach for the treatment of ASA-induced allergic and inflammatory disorders such as ASA intolerance and FDEIA.

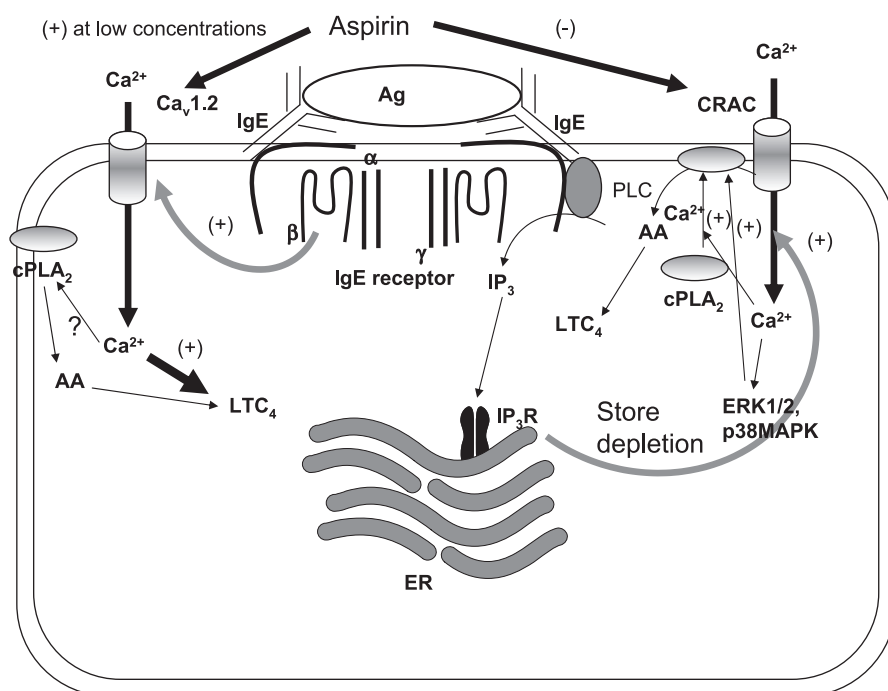


Fig. 6. Model for the classic and the novel FcεRI-mediated LTC₄ synthesis pathways in mast cells. In the traditional view, cross-linking of FcεRI on mast cells results in the activation of SOCs, including CRAC channels via the FcεRIγ-Syk-PLCγ-IP₃ pathway, which leads to [Ca²⁺]_i increase. This [Ca²⁺]_i increase in concert with ERK1/2 and/or p38MAPK activation cause increased LTC₄ synthesis via cPLA₂ activation. This SOC-mediated LTC₄ synthesis is strictly dependent on mitochondrial polarization, probably because SOC activation requires normal mitochondrial functions. Our recent studies (24, 25) demonstrate that FcεRI cross-linking also stimulates LTCCs such as Ca_v1.2 via the FcεRIβ in a store-independent manner, and [Ca²⁺]_i increase through these Ca²⁺ channels facilitates LTC₄ synthesis independently of ERK1/2, p38MAPK, and mitochondrial polarization. ASA and SA at low concentrations selectively facilitate this novel LTC₄ synthesis pathway.

Acknowledgments

We would like to thank Dr. K. Togo and Prof. T. Ochiai at the Department of Dermatology, Surugadai Nihon University Hospital and Prof. T. Terui at the Department of Dermatology, Nihon University School of Medicine for their collaborations. We thank Dr. T. Yoshimaru and T. Inoue at the Division of Molecular Cell Immunology and Allergology, Nihon University Graduate School of Medical Science for their technical assistance. We thank the National Institute of Health Sciences (Japan Collection of Research Biosources) for providing RBL-2H3 (Cell number JCRB0023). This work was supported in part by the 50th anniversary medical department foundation from Nihon University (2006–2007); a Grant-in-Aid for Scientific Research (C) (No. 19591330, to T.O.); and a Grant-in-Aid “High-Tech Research Center” Project (2002–2006) for Private Universities matching fund subsidy from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

References

- Grattan CE. Aspirin sensitivity and urticaria. *Clin Exp Dermatol*. 2003;28:123–127.
- Ying S, Corrigan CJ, Lee TH. Mechanisms of aspirin-sensitive asthma. *Allergol Int*. 2004;53:111–119.
- Morita E, Kunie K, Matsuo H. Food-dependent exercise-induced anaphylaxis. *J Dermatol Sci*. 2007;47:109–117.
- Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat New Biol*. 1971;231:232–235.
- Wu KK. Aspirin and other cyclooxygenase inhibitors: new therapeutic insights. *Semin Vasc Med*. 2003;3:107–112.
- Kopp E, Ghosh S. Inhibition of NF-κB by sodium salicylate and aspirin. *Science*. 1994;265:956–959.
- Román J, de Arriba AF, Barrón S, Michelena P, Giral M, Merlos M, et al. UR-1505, a new salicylate, blocks T cell activation through nuclear factor of activated T cells. *Mol Pharmacol*. 2007;72:269–279.
- Mortaz E, Redegeld FA, Nijkamp FP, Engels F. Dual effects of acetylsalicylic acid on mast cell degranulation, expression of cyclooxygenase-2 and release of pro-inflammatory cytokines. *Biochem Pharmacol*. 2005;69:1049–1057.
- O’Sullivan S, Dahlén B, Dahlén SE, Kumlin M. Increased urinary excretion of the prostaglandin D₂ metabolite 9α, 11β-prostaglandin F₂ after aspirin challenge supports mast cell activation in aspirin-induced airway obstruction. *J Allergy Clin Immunol*. 1996;98:421–432.
- Mita H, Endoh S, Kudoh M, Kawagishi Y, Kobayashi M, Taniguchi M, et al. Possible involvement of mast-cell activation in aspirin provocation of aspirin-induced asthma. *Allergy*. 2001;56:1061–1067.
- Wang XS, Wu AY, Leung PS, Lau HY. PGE₂ suppresses excessive anti-IgE induced cysteinyl leukotrienes production in mast cells of patients with aspirin exacerbated respiratory disease. *Allergy*. 2007;62:620–627.
- Bae JS, Kim SH, Ye YM, Yoon HJ, Suh CH, Nahm DH, et al. Significant association of FcεRIα promoter polymorphisms with aspirin-intolerant chronic urticaria. *J Allergy Clin Immunol*. 2007;119:449–456.
- Togo K, Suzuki Y, Yoshimaru T, Inoue T, Terui T, Ochiai T, et al. Aspirin and salicylates modulate IgE-mediated leukotriene secretion in mast cells through a dihydropyridine receptor-mediated Ca²⁺ influx. *Clin Immunol*. 2009;131:145–156.
- Stevenson DD, Hankammer MA, Mathison DA, Christiansen SC, Simon RA. Aspirin desensitization treatment of aspirin-sensitive patients with rhinosinusitis-asthma: long-term outcomes. *J Allergy Clin Immunol*. 1996;98:751–758.
- Leslie CC. Properties and regulation of cytosolic phospholipase A₂. *J Biol Chem*. 1997;272:16709–16712.

- 16 Nemenoff RA, Winitz S, Qian NX, Van Putten V, Johnson GL, Heasley LE. Phosphorylation and activation of a high molecular weight form of phospholipase A₂ by p42 microtubule-associated protein 2 kinase and protein kinase C. *J Biol Chem.* 1993;268: 1960–1964.
- 17 Rosado JA, Sage SO. Role of the ERK pathway in the activation of store-mediated calcium entry in human platelets. *J Biol Chem.* 2001;276:15659–15665.
- 18 Börsch-Haubold AG, Bartoli F, Asselin J, Dudler T, Kramer RM, Apitz-Castro R, et al. Identification of the phosphorylation sites of cytosolic phospholipase A₂ in agonist-stimulated human platelets and HeLa cells. *J Biol Chem.* 1998;273:4449–4458.
- 19 Hefner Y, Börsch-Haubold AG, Murakami M, Wilde JI, Pasquet S, Schieltz D, et al. Serine 727 phosphorylation and activation of cytosolic phospholipase A₂ by MNK1-related protein kinases. *J Biol Chem.* 2000;275:37542–37551.
- 20 Gijón MA, Leslie CC. Regulation of arachidonic acid release and cytosolic phospholipase A₂ activation. *J Leukoc Biol.* 1999; 65:330–336.
- 21 Zweifach A, Lewis RS. Calcium-dependent potentiation of store-operated calcium channels in T lymphocytes. *J Gen Physiol.* 1996;107:597–610.
- 22 Chang WC, Parekh AB. Close functional coupling between Ca²⁺ release-activated Ca²⁺ channels, arachidonic acid release, and leukotriene C₄ secretion. *J Biol Chem.* 2004;279:29994–29999.
- 23 Chang WC, Nelson C, Parekh AB. Ca²⁺ influx through CRAC channels activates cytosolic phospholipase A₂, leukotriene C₄ secretion, and expression of c-fos through ERK-dependent and -independent pathways in mast cells. *FASEB J.* 2006;20:2381–2383.
- 24 Suzuki Y, Yoshimaru T, Inoue T, Ra C. The high-affinity immunoglobulin E receptor (FcεRI) regulates mitochondrial calcium uptake and a dihydropyridine receptor-mediated calcium influx in mast cells: role of the FcεRIβ chain immunoreceptor tyrosine-based activation motif. *Biochem Pharmacol.* 2008;75:1492–1503.
- 25 Yoshimaru T, Suzuki Y, Inoue T, Ra C. L-type Ca²⁺ channels in mast cells: activation by membrane depolarization and distinct roles in regulating mediator release from store-operated Ca²⁺ channels. *Mol Immunol.* 2009;46:1267–1277.