



Regulation of mast cell activation by extracellular vesicles in cow's milk casein-induced allergic responses

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Abstract

Background Food allergy is a hypersensitive immune reaction to food proteins including cow's milk protein. Extracellular vesicles (EVs), such as exosomes, are newly discovered intercellular conveyors of functional molecular mechanisms and mediated intercellular interactions involving mast cells and are particularly relevant to allergy.

Objective In this study, we investigated whether cow's milk casein-induced allergy (CIA)-derived EVs can modulate mast cell activation.

Results EVs in CIA mice and control mice were isolated using the ultracentrifugation method and the isolated EVs were quantified using BCA analysis. CIA responses were determined through changes in body temperature and systemic symptom score. The number of EVs was higher in CIA-derived EVs compared to normal EVs. EVs marker proteins such as CD63 and CD9 were elevated in CIA-derived EVs. The levels of EV-associated cytokines such as IL-6, IL-8, and TNF- α were significantly elevated in CIA-derived EVs. In addition, CIA-derived EVs significantly induced degranulation via Lyn kinase activation in mouse bone marrow-derived mast cells and human mast cells.

Conclusion Our results demonstrate that CIA-derived EVs can induce a reaction similar to cow's milk allergic reaction via mast cell activation. These results provide an insight into the pathology of cow's milk allergy and a potential therapeutic approach through targeting EV release and/or uptake.

Keywords Extracellular vesicles (EVs) · Food allergy · Cow's milk allergy (CMA) · Mast cells · Casein-induced allergy (CIA)

Introduction

Anaphylaxis is an acute systemic allergic reaction that is potentially fatal (Sampson et al. 2006). Allergenic foods such as milk, peanut, egg, soy, wheat, and tree nuts are the major triggers of anaphylaxis, accounting for 33%–56% of all adult cases and up to 81% of all children cases

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(Ben-Shoshan and Clarke 2011; Cianferoni et al. 2001; Wang and Sampson 2007). One of the best known for food allergies is the cow's milk allergy (CMA), which account for 2.5–5% of all allergic diseases, and which is substantially associated with anaphylaxis and death (Krishnamurthy et al. 2012; Li et al., 1999; Schouten et al. 2012; Shreffler et al. 2009). Worldwide, incidences of self-reported adverse reactions to CMA are higher than the medically confirmed diagnoses, in both children and adults (Fiocchi et al. 2010). CMA is an abnormal immune-mediated response to the presence of certain protein in cow's milk. Casein (approximately 80%) and whey proteins (approximately 20%) are the major cow's milk protein, and the major allergenic proteins have been identified within these two groups (Schulmeister et al. 2009; van Esch et al. 2013). The cow's milk allergy can be characterized by two causal aspects, first being IgE-mediated ("acute onset"), the second being non-IgE-mediated ("delayed onset"), or both, of which the first being the most common and fatal (Walsh et al. 2016). In the case of IgE-mediated CMA patients, secretion of mast cell intracellular granules and mast cell activation following antigen-IgE-FcεRI signaling cause the early symptoms of CMA (Jo et al. 2014).

Mast cells (MCs) are the major effector cells that mediate allergic responses (Galli et al. 2008; Kawakami et al. 2009; Metz et al. 2007). After stimulation by an antigen, mast cells secrete a large number of granules containing histamine and other preformed inflammatory mediators, and extracellular vesicles (EVs) such as exosomes (40–150 nm in diameter) and microvesicles (50–1000 nm in diameter) (Moon et al. 2014).

EVs, such as exosomes, are membrane vesicles secreted from various cells (Cho et al. 2018b) including mast cells (Al-Nedawi et al. 2005; Carroll-Portillo et al. 2012; Skokos et al. 2002). The presence of exosomes in various biological matrix, including complex fluid or tissue, implies that EV can have a biological function in regulation of immune system (Carroll-Portillo et al. 2012). EVs are key mediators of intercellular communication in physiological and pathological responses. In particular, exosomes are known to be involved in modulation of immune responses as a key mediator (Nazimek et al. 2016). As a result, interest about exosomes has recently been rising as a potential therapeutic target for several diseases.

However, the pathogenical and physiological roles of exosomes in cow's milk allergy have not been completely understood. In this study, we investigated whether casein-induced allergic response induced the production of EVs and exosomal cytokines such as IL-6, IL-8, and TNF-α using a mouse model. Furthermore, we investigated whether EVs from casein-induced allergy had a direct role in modulation of mast cell activation *in vitro*.

Materials and methods

Induction of casein-mediated food allergy in mice

Induction of casein-mediated food allergy in mice was performed as previously described (Kim et al. 2016). Briefly, all experiments were performed in accordance with the guidelines and protocols of the Institutional Animal Care and Use Committee (IACUC) at Andong National University. The animals were housed at 20 ± 2 °C with 12 h light/dark cycles and a relative humidity of $50 \pm 5\%$ under filtered, pathogen-free air, with food and water available *ad libitum*, and were stabilized for at least 2 weeks prior to each experiment. The 6-month-old male C57BL/6J mice were fasted overnight, while water was provided *ad libitum*. Female mice were sensitized on days 0 and 7 by oral administration (p.o.) in 250 μL PBS containing 1 mg of casein (Sigma-Aldrich, St. Louis, MO) and cholera toxin 10 μg (CT, List Biological Laboratories, Inc., Campbell, CA). After 1 week, oral boost of mice was performed for four times with 5 mg of casein containing cholera toxin 10 μg at days 14–21. After 7 days, allergic responses of mice were induced by oral administration 250 μL of PBS containing 50 mg of casein. Rectal temperature as severity of food allergy was measured by digital thermometer TESTO 925 immediately after induction. Symptom scores were as follows: 0, no symptoms; 1, rubbing and scratching around the nose and head; 2, puffiness around the mouth and eyes; 3, labored breathing and cyanosis around the mouth and tail; 4, tremor and no activity after prodding; 5, death.

Measurement of casein-specific antibody in serum

Casein-specific IgA, IgG1, IgG2a, and IgE levels from mice serum were performed by ELISA as previously described (Kim et al. 2016). In brief, 50 μg of casein/well in buffer (0.1 M sodium carbonate, pH 9.5) was coated on microtiter plate and incubated for 12 h at 4 °C. Wells were washed twice with PBS and then incubated with 5% FBS in PBS for 2 h. After blocking, serum samples of mice were diluted 1:10 in PBS. Diluted samples were added 100 wells and incubated for 2 h at room temperature. Wells were washed three times with blocking buffer. After washing, 2 μg biotin-labeled rat anti-mouse IgE, IgG1, IgG2a, or IgA (BD Biosciences, San Jose, CA) were added to the well and incubated for 1 h at room temperature. After washing with the blocking buffer, streptavidin-HRP (BD Biosciences, San Jose, CA) was added to the plate well and incubated for 30 min at room temperature which was latter replaced with a substrate solution (TMB; GenDEPOT, Barker, TX, USA). The absorbance of samples was measured at 450 nm.

EVs' isolation

Plasma exosomes were isolated as our previous study (Cho et al. 2018a; Kim et al. 2018). In brief, EVs from sera with casein-induced allergy mice group and normal mice group were prepared using ExoQuick solution (System Bioscience) following the manufacturer's instructions. The final pellet sample of EVs obtained by ExoQuick-method was stored at 4 °C or at −20 °C, and subsequently used for immunoblotting, ELISA, and β -hexosaminidase release assay.

Nanoparticle tracking analysis (NanoSight™)

The number and size of isolated exosomes were measured by nanoparticle tracking analysis (NTA) using a NanoSight NS300 system (NanoSight, Amesbury, UK) (Cho et al. 2018a; Kim et al. 2018). The instrument was calibrated with 100 nm polystyrene beads (Thermo-Fisher Scientific, Fremont, CA) before being used. NTA software was used to measure the concentration of nanoparticles (particles/mL). The Batch Process included in the NTA software was used to integrate the three technical measurements of each sample.

Culture of murine bone marrow-derived mast cells (BMMCs) and human mast cell lines (LAD2 cells)

Bone marrow-derived mast cells (BMMCs) were isolated from mouse bone marrows and cultured for 4–8 weeks in RPMI 1640 containing 2 mM L-glutamine, 0.1 mM non-essential amino acids, 100 IU/mL penicillin, 100 mg/mL streptomycin, 1 mM sodium pyruvate, 1 mM HEPES, 10% FBS, and 10 ng/mL IL-3. LAD2 cells were maintained in complete StemPro-34 SFM Complete Medium supplemented with 100 ng/mL human recombinant SCF, L-glutamine (2 mM), penicillin (100 IU/mL), and streptomycin (100 mg/mL). The BMMCs and LAD2 cells were maintained at 37 °C and 5% CO₂.

Immunoblot analysis

For immunoblot analyses of serum-derived EVs, purified EVs' preparations were lysed in 100 μ L of RIPA buffer (20 mM Tris-HCl, 150 mM NaCl, 1 mM Na₂EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM β -glycerophosphate, 1 mM Na₃VO₄, and 1 μ g/mL leupeptin). EV lysates were kept on ice for 10 min and dissolved in 3X Laemmli buffer. Equal amounts (10 μ L) from different samples were separated by SDS-PAGE using 4–12% gels and then transferred onto nitrocellulose membranes. The membranes were blocked with 5% BSA in Tris-buffered saline for 1 h, and then incubated with specific primary antibodies overnight at 4 °C. Antibodies used were against anti-CD63 (1:10,000

dilutions; Santa Cruz Biotechnology, Dallas, TX, USA) and anti-CD9 (1:10,000 dilutions; Santa Cruz Biotechnology, Dallas, TX, USA). Relative protein images were determined using HRP-conjugated secondary antibodies and ECL substrates (Thermo-Fisher Scientific). The densitometric intensities of the immunoreactive bands were quantified using ImageJ software (National Institutes of Health).

Measurement of Exosomal IL-6, IL-8, and TNF- α by ELISA

As mentioned previously, the sample of isolated EVs from sera with each group was used. Isolated EVs (100 μ g) were lysed in RIPA buffer and the amounts of exosomal IL-6, IL-8, and TNF- α were measured using OptEIA™ ELISA kits (BD Biosciences, San Jose, CA, USA) according to the manufacturer's protocol.

Measurement of degranulation in MCs

The LAD2 human mast cell line, and primary mouse BMMCs were cultured in 24-well plates (2×10^5 cells per well) and maintained in growth media (with EV-depleted 10% FBS) for 24 h at 37 °C under 5% CO₂. At 80% confluency, cells were washed with HEPES-BSA buffer (10 mM HEPES, pH 7.4, 137 mM NaCl, 2.7 mM KCl, 0.4 mM Na₂HPO₄, 5.6 mM glucose, 1.8 mM CaCl₂, 1.3 mM MgSO₄, and 0.04% BSA). The cells were treated with the indicated concentration of EVs (10 μ g, 30 μ g, 100 μ g, or 300 μ g) in HEPES-BSA buffer for 2 h. The degree of MC degranulation is expressed as the percent of the activity of β -hexosaminidase secreted out of the cells compared to the activity of total β -hexosaminidase. The OD value was analyzed on spectrophotometers at 405 nm.

Statistical analysis and other methods

Statistical significance was determined using two-tailed *t* test; ANOVA and Dunnet's multiple comparison post-test were used to compare the means of multiple groups. All analyses were performed using Prism 7 software (GraphPad Software Inc., La Jolla, CA, USA). Statistical significance was indicated as follows: **P* < 0.05, ***P* < 0.01, and ****P* < 0.001.

Results

Estimation of allergic response in casein-induced allergy (CIA) mice

Casein-induced food allergic responses were determined using changes in body temperature, systemic symptom score, and casein-specific antibodies (Fig. 1). Rectal temperature was significantly lower in CIA mice compared to PBS-treated normal mice, and allergic symptom score was significantly elevated in CIA mice (Fig. 1a). Furthermore, casein-specific antibodies (IgE, IgG1, IgG2a, and IgA) levels were significantly higher in CIA mice compared to normal mice (Fig. 1b).

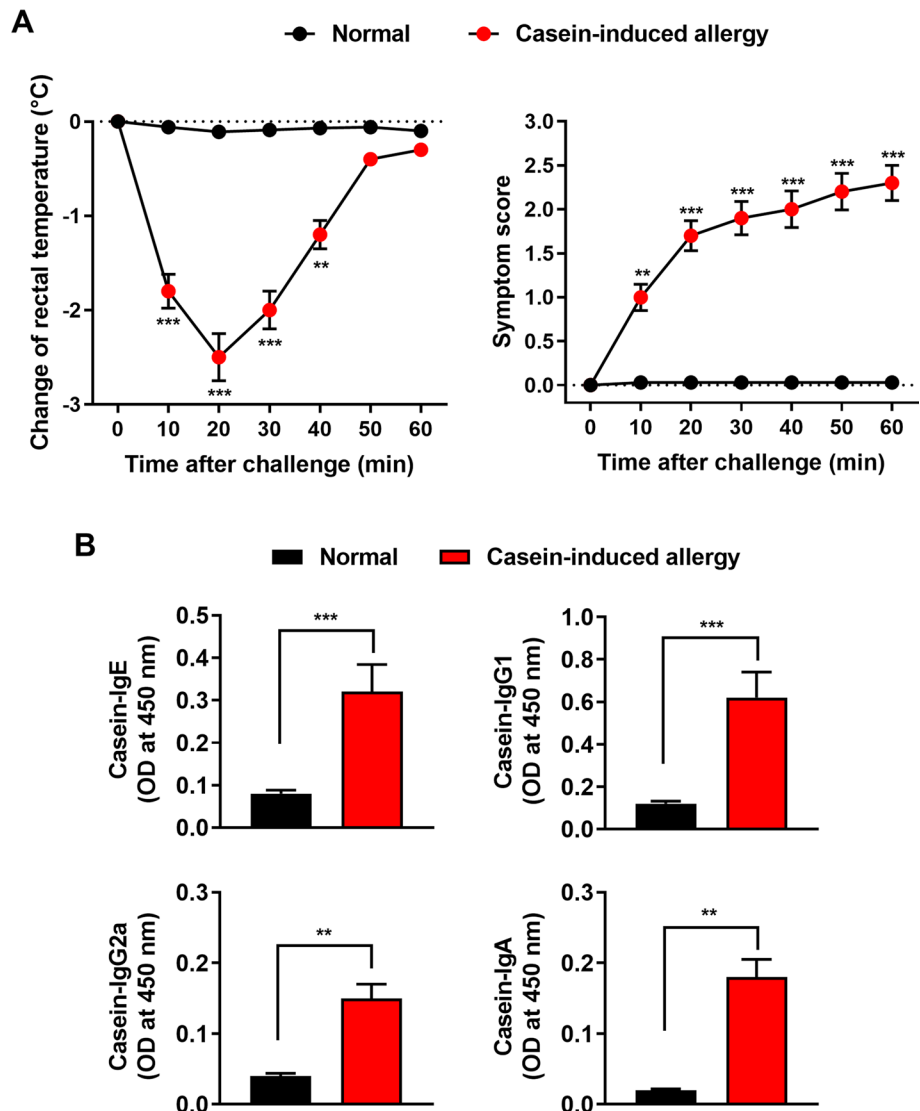
Characterization of CIA-derived EVs

First, EVs' concentration was higher in CIA mice than in PBS-treated normal mice (Fig. 2a). Nanoparticle tracking analysis in PBS-treated normal mice-derived EVs and CIA-derived EVs showed a majority of small-sized particle distribution (30–200 nm) (Fig. 2b). Notably, the average sizes of PBS-treated normal mice-derived EVs and CIA-derived EVs were 102.5 nm and 104.4 nm, respectively (Fig. 2b). Our results demonstrated that casein-induced food allergic responses release small-sized EVs.

Exosome marker proteins and pro-inflammatory cytokines in CIA-derived EVs

Several studies showed that tetraspanins such as CD9, CD63, CD81, etc. are highly conserved in EVs, so various researchers are using the CD9, CD63, and CD81 as the EVs marker

Fig. 1 Casein-induced allergy (CIA) mice model. **a** Changes in rectal temperatures and symptom scores were evaluated as severity of CIA. **b** The amount of casein-specific IgE, IgG1, IgG2a, and IgA antibodies were assessed by ELISA as described in “Materials and Methods”. Data are mean $\pm$ SEM ($n = 10$). The statistical differences between the normal and CIA groups are shown. ** $P < 0.01$, *** $P < 0.001$



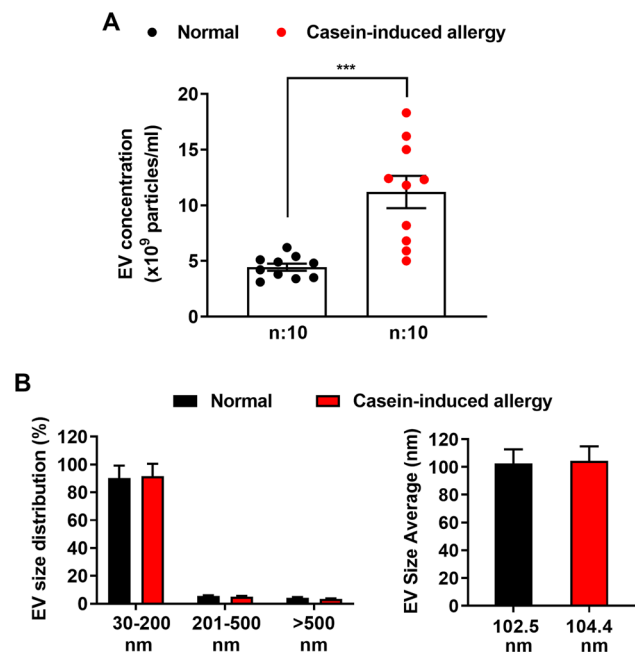
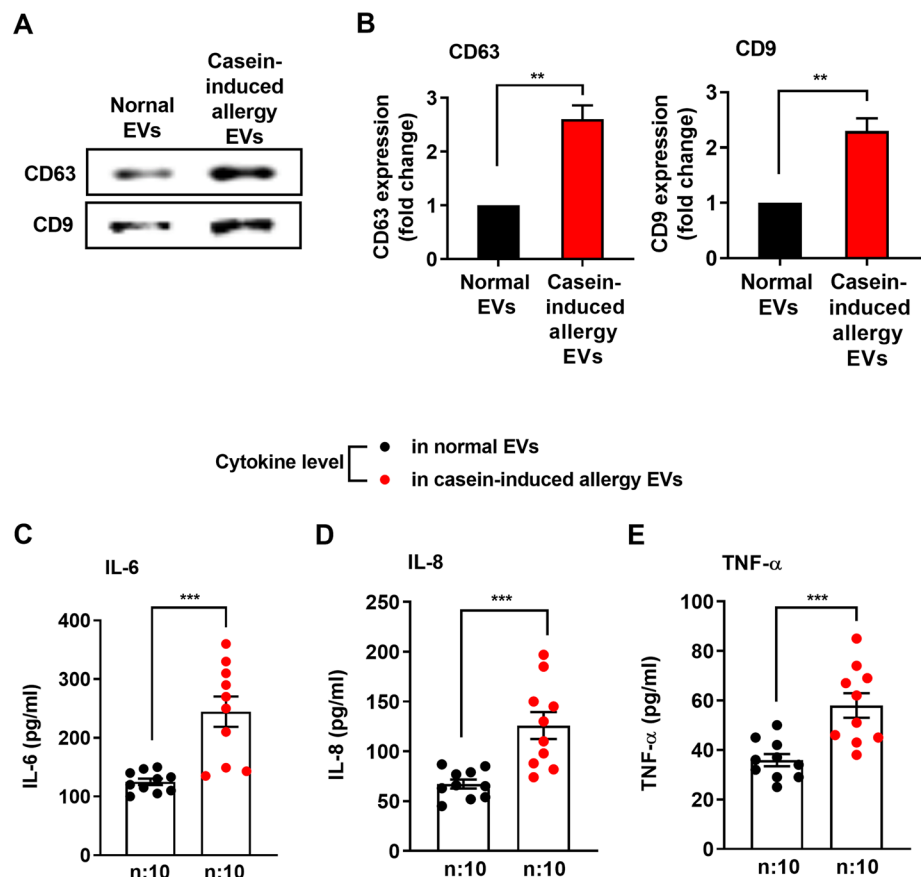


Fig. 2 Characterization of EVs secreted in CIA mice model. **a** Concentration of EVs in the normal and CIA groups was measured by Nanosight analysis. **b** Analysis of the size distribution (%) and average (avg.) diameters (nm) of normal EVs and CIA EVs. Data are mean $\pm$ SEM ($n=10$). The statistical differences between the normal and casein-induced allergy groups are shown. *** $P < 0.001$

Fig. 3 The elevation of exosomal marker proteins and cytokines in CIA-derived EVs. **a, b** Representative immunoblot images of exosome markers such as CD63 and CD9. Densitometric quantitation of the immunoblots for exosome markers relative to normal EVs is shown. **c–e** The amounts of **c** IL-6; **d** IL-8; and **e** TNF- α in normal EVs and CIA EVs. Data are mean $\pm$ SEM ($n=10$). The statistical differences between the normal and CIA groups are shown. ** $P < 0.01$, *** $P < 0.001$



(Andreu et al. 2014; Kalluri et al. 2020; Stoolvogel et al. 2002). On the basis of these investigations, we performed immunoblot analysis to examine the presence of exosomes in CIA-induced mice (Fig. 3a, b). The expression levels of CD63 and CD9 in CIA-induced mice serum were higher than in normal mice (Fig. 3a, b).

CIA-induced elevated levels of EVs markers were considered to be related to the inflammatory environment on Cow's milk allergy. As previous reported (Paajanen et al. 2006), the patients with Cow's milk allergy exhibit higher levels of IL-6, IL-8, and TNF- α (Paajanen et al. 2006; Caubet et al. 2017). Thus, we also evaluated the amounts of cytokines such as IL-6, IL-8, and TNF- α in EVs from CIA-induced mice. The levels of IL-6, IL-8, and TNF- α in CIA EVs were higher than in Normal-EVs when equal amounts of protein lysates were subjected (Fig. 3c–e). Taken together, these results suggest that CIA-derived EVs might stimulate activation of immune cells relatively associated with inflammatory response.

CIA-derived EVs promote the activation of mast cells

We investigated the potential of altering the function of mast cell involved in allergic responses by CIA-derived EVs. Activation of mast cells was determined by measurement

of degranulation. Our results showed that CIA-derived EVs significantly induced the degranulation in mouse bone marrow-derived mast cells (Fig. 4a) and human mast cells (Fig. 4b). These data indicate that CIA-derived EVs can induce food allergy response through mast cells activation, supporting the functional roles of EVs in mast cell activation after uptake of CIA-derived EVs by mast cells.

CIA-derived EVs induce mast cells degranulation via Lyn kinase activation

We next investigated the effects of CIA-derived EVs on the activation of src kinase family that are required for activation of mast cells (Gilfillan and Rivera 2009; Lee et al. 2011). Lyn was activated by CIA-derived EVs (Fig. 5a). However, the activities of Fyn and Fgr (Fig. 5b, c) were not affected. Our results demonstrated that CIA-derived EVs significantly induced mast cell degranulation via Lyn kinase activation.

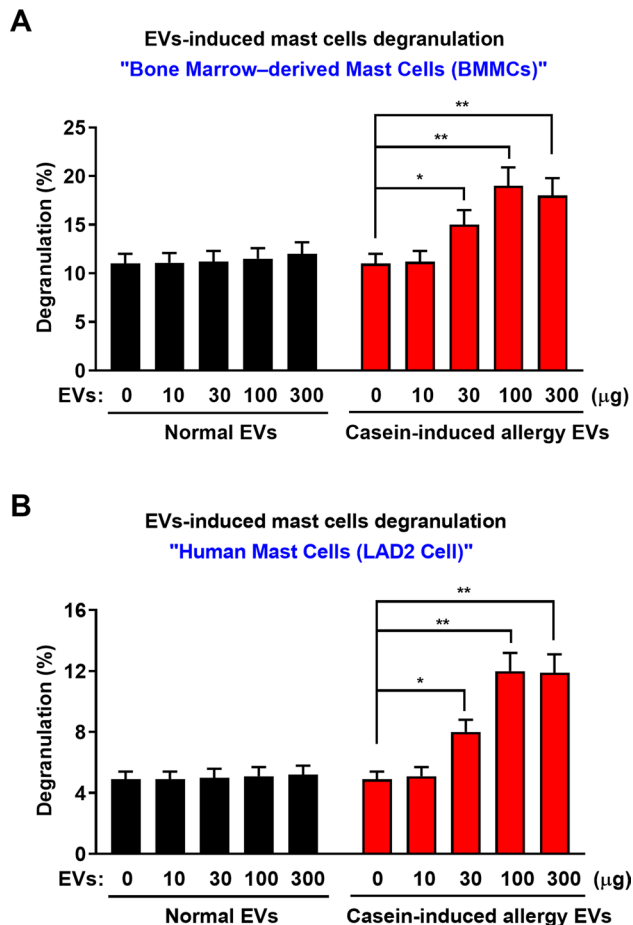


Fig. 4 Mast cell degranulation by CIA-derived EVs. **a**, **b** Confluent mouse bone marrow-derived mast cells and human mast cells were treated with 0, 10, 30, 100, and 300 µg of normal EVs or CIA EVs for 2 h. The representative degranulation (%) of **a** mouse bone marrow-derived mast cells and **b** human mast cells by CIA-derived EVs. Data are mean $\pm$ SEM ($n = 10$). * $P < 0.05$, ** $P < 0.01$

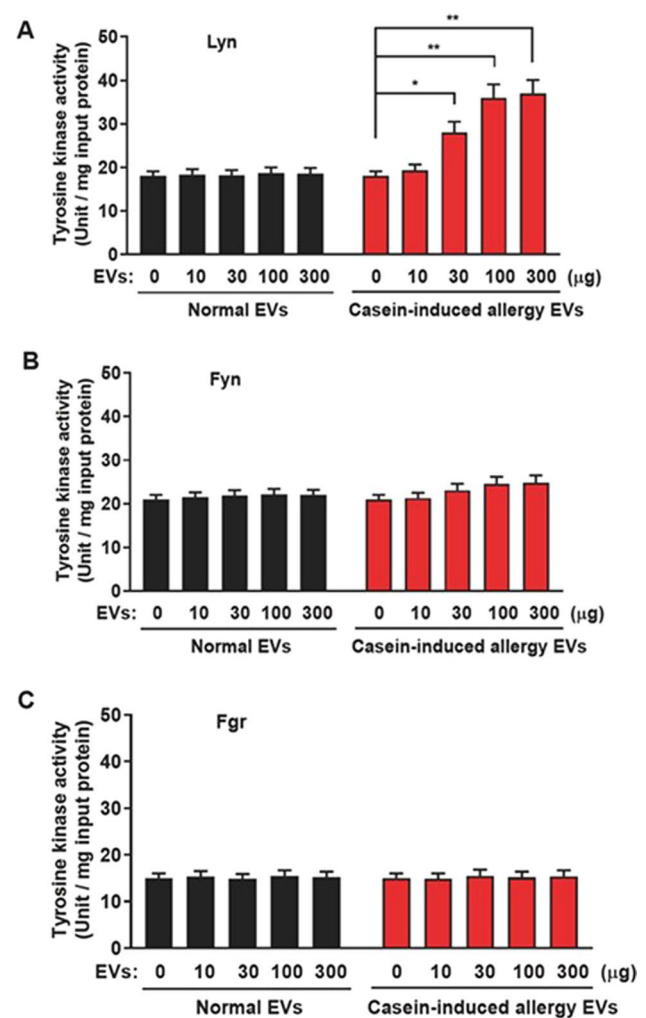


Fig. 5 Activation of tyrosine kinases by CIA-derived EVs. **a** Lyn, **b** Fyn, and **c** Fgr were immunoprecipitated from 1 mg of protein of whole-cell lysates in normal EVs or CIA EVs treated BMMCs. The activities of immunoprecipitates were measured using the ELISA-based Tyrosine Kinase Assay Kit. Data are mean $\pm$ SEM ($n = 5$). * $P < 0.05$, ** $P < 0.01$

Discussion

In the current study, we demonstrated that casein-induced allergy response in mice released small-sized EVs. In addition, our results showed that CIA-derived EVs contained high amounts of exosome marker proteins (CD63 and CD9) and pro-inflammatory cytokines such as IL-6, IL-8, and TNF- α . Notably, the degranulation of mouse bone marrow-derived mast cells and human mast cells was modulated by CIA-derived EVs, suggesting that CIA-derived EVs may play a critical role in the mast cell activation.

Mast cells are positioned in connective tissues, such as under the skin, near blood vessels, and lymphatic vessels, lungs, and the intestines. These distributed MCs have an essential role in allergic disorders, such as atopic asthma,

allergic rhinitis, atopic dermatitis, and anaphylaxis, which begins by secreting bioactive molecules including several proteases and biogenic amines (e.g., histamine and serotonin) (Park et al. 2019).

EVs, including exosomes, are released by a number of cell types and contain cell-type-specific and selectively packaged proteins, mRNAs, and small RNAs that are transported stably in circulation and taken up by recipient cells in a process believed to be non-random. Cultured mast cells are known to secrete EVs constitutively or during degranulation (Admyre et al. 2008; Groot Kormelink et al. 2016; Skokos et al. 2003; Skokos et al. 2002). Previously, we demonstrated that patients with systemic mastocytosis (SM) have high concentrations of small EVs in circulation with characteristic mast cell markers that have the potential of altering the phenotype of non-hematopoietic cells (Kim et al. 2018). In the present study, we verified that CIA-induced mice had higher concentrations of small-sized EVs, which promote mast cell activation. Also, we showed that EVs isolated from 100 μ L of serum in CIA-induced mice contained high levels of IL-6, IL-8, and TNF- α , and subsequently, the mast cell may have been activated.

Elevated levels of pro-inflammatory cytokines, such as IL-6 and IL-8, accelerate the development of hyper-inflammatory response (Volpin et al. 2014). Similarly, our results showed that IL-6 and IL-8 levels were increased in CIA-derived EVs compared to normal EVs. A proteomic analysis of mast cell-derived exosomes has been reported that mast cell-derived exosomes contain TNF- α , angiotensinogen and prothrombin (Al-Nedawi et al. 2005). In this study, we found out that TNF- α was increased in CIA-derived EVs compared to normal EVs.

EVs may directly activate the recipient cell by acting as signaling complexes and can play a crucial role in inducing T- and B- lymphocyte proliferation as a constituent of immunomodulatory network (Skokos et al. 2003). A previous study also reported that mast cells and mast cell-derived exosomes preferentially induce Th1-type responses as evidenced by the production of IL-2, IFN- γ , and IL-12 (Tkaczyk et al. 2000). Our results suggest that treatment of CIA-derived EVs to mast cell results in uptake of various substances and induce mast cell degranulation, a process of mast cell activation. Taken together, CIA-derived EVs similar to cow's milk can induce allergic responses via mast cells activation.

Based on our study, we report that EV concentration in CIA-induced mice was significantly higher than in normal mice groups, and that transferring CIA-derived EVs including IL-6, IL-8, and TNF- α to mast cells promotes mast cell activation. Our present study provides an insight into the pathology of Cow's milk allergy and a potential therapeutic approach through targeting EV release and/or uptake. Future studies will be required to elucidate the molecular

mechanisms on how CIA-derived EVs regulate mast cell activation.

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Author contributions Conceptualization, D-KK and Y-EC; methodology, validation, formal analysis, and data curation, D-KK, H-WK, K-YM, J-HH, D-HK, Y-GN, J-IK, I-SK, and M-CB; project administration, D-KK; funding acquisition Y-EC, M-CB, J-HL, and D-KK; writing original draft preparation, D-KK, Y-EC, and H-WK; writing review and editing, all authors; visualization, D-KK and Y-EC.

Declarations

Conflict of interest Young-Eun Cho, Hyun-Woo Kim, Keun Young Min, Jin-Hyeon Hwang, Dong-Ha Kim, Jee-In Kim, Guo Nan Yin, Jae-Hwan Lim, In-Sook Kwun, Moon-Chang Baek, and Do-Kyun Kim declare that they have no conflicts of interest.

Human and animal rights The article does not contain any studies with human. All animal experiments were performed in accordance with the guidelines and protocols of the Institutional Animal Care and Use Committee (IACUC) at Andong National University.

References

- Admyre C et al (2008) Exosomes—nanovesicles with possible roles in allergic inflammation. *Allergy* 63:404–408
- Al-Nedawi K, Szemraj J, Cierniewski CS (2005) Mast cell-derived exosomes activate endothelial cells to secrete plasminogen activator inhibitor type 1. *Arterioscler Thromb Vasc Biol* 25:1744–1749
- Andreu Z, Yáñez-Mó M (2014) Tetraspanins in extracellular vesicle formation and function. *Front Immunol* 5:442
- Ben-Shoshan M, Clarke AE (2011) Anaphylaxis: past, present and future. *Allergy* 66:1–14
- Carroll-Portillo A et al (2012) Mast cell synapses and exosomes: membrane contacts for information exchange. *Front Immunol* 3:46
- Caubet JC et al (2017) Humoral and cellular responses to casein in patients with food protein-induced enterocolitis to cow's milk. *J Allergy Clin Immunol* 39:572–583
- Cho YE et al (2018a) Exogenous exosomes from mice with acetaminophen-induced liver injury promote toxicity in the recipient hepatocytes and mice. *Sci Rep* 8:16070
- Cho YE, Song BJ, Akbar M, Baek MC (2018b) Extracellular vesicles as potential biomarkers for alcohol- and drug-induced liver injury and their therapeutic applications. *Pharmacol Ther* 187:180–194
- Cianferoni A et al (2001) Clinical features of acute anaphylaxis in patients admitted to a university hospital: an 11-year retrospective review (1985–1996). *Ann Allergy Asthma Immunol* 87:27–32

- Fiocchi A et al (2010) Diagnosis and rationale for action against cow's milk allergy (DRACMA): a summary report. *J Allergy Clin Immunol* 126:1119–1128 e12
- Galli SJ, Tsai M, Piliponsky AM (2008) The development of allergic inflammation. *Nature* 454:445–454
- Gilfillan AM, Rivera J (2009) The tyrosine kinase network regulating mast cell activation. *Immunol Rev* 228:149–169
- Groot Kormelink T et al (2016) Mast cell degranulation is accompanied by the release of a selective subset of extracellular vesicles that contain mast cell-specific proteases. *J Immunol* 197:3382–3392
- Jo J, Garssen J, Knippels L, Sandalova E (2014) Role of cellular immunity in cow's milk allergy: pathogenesis, tolerance induction, and beyond. *Mediators Inflamm* 214:249784
- Kalluri R, Lebleu VS (2020) The biology, function and biomedical applications of exosomes. *Science* 367:eaau6977
- Kawakami T et al (2009) Mast cells in atopic dermatitis. *Curr Opin Immunol* 21:666–678
- Kim AR et al (2016) Mesenteric IL-10-producing CD5+ regulatory B cells suppress cow's milk casein-induced allergic responses in mice. *Sci Rep* 6:19685
- Kim DK et al (2018) Mastocytosis-derived extracellular vesicles exhibit a mast cell signature, transfer KIT to stellate cells, and promote their activation. *Proc Natl Acad Sci USA* 115:E10692–E10701
- Krishnamurthy D et al (2012) Monitoring neutrophils and platelets during casein-induced anaphylaxis in an experimental BALB/c mouse model. *Clin Exp Allergy* 42:1119–1128
- Lee JH et al (2011) The Src family kinase Fgr is critical for activation of mast cells and IgE-mediated anaphylaxis in mice. *J Immunol* 187:1807–1815
- Li XM et al (1999) A murine model of IgE-mediated cow's milk hypersensitivity. *J Allergy Clin Immunol* 103:206–214
- Metz M et al (2007) Mast cells in the promotion and limitation of chronic inflammation. *Immunol Rev* 217:304–328
- Moon TC, Befus AD, Kulka M (2014) Mast cell mediators: their differential release and the secretory pathways involved. *Front Immunol* 5:569
- Nazimek K, Bryniarski K, Askenase PW (2016) Functions of exosomes and microbial extracellular vesicles in allergy and contact and delayed-type hypersensitivity. *Int Arch Allergy Immunol* 171:1–26
- Paajanen L et al (2006) Intestinal cytokine mRNA expression in delayed-type cow's milk allergy. *J Pediatr Gastroenterol Nutr* 43:470–476
- Park YH et al (2019) WZ3146 inhibits mast cell Lyn and Fyn to reduce IgE-mediated allergic responses in vitro and in vivo. *Toxicol Appl Pharmacol* 383:114763
- Sampson HA et al (2006) Second symposium on the definition and management of anaphylaxis: summary report—second national institute of allergy and infectious disease/food allergy and anaphylaxis network symposium. *J Allergy Clin Immunol* 117:391–397
- Schouten B et al (2012) A potential role for CD25+ regulatory T-cells in the protection against casein allergy by dietary non-digestible carbohydrates. *Br J Nutr* 107:96–105
- Schulmeister U et al (2009) Cloning, expression, and mapping of allergenic determinants of alphaS1-casein, a major cow's milk allergen. *J Immunol* 182:7019–7029
- Shreffler WG et al (2009) Association of allergen-specific regulatory T cells with the onset of clinical tolerance to milk protein. *J Allergy Clin Immunol* 123:43–527
- Skokos D, Goubran-Botros H, Roa M, Mecheri S (2002) Immunoregulatory properties of mast cell-derived exosomes. *Mol Immunol* 38:1359–1362
- Skokos D et al (2003) Mast cell-derived exosomes induce phenotypic and functional maturation of dendritic cells and elicit specific immune responses in vivo. *J Immunol* 170:3037–3045
- Stoorvogel W, Kleijmeer MJ, Geuze HJ, Raposo G (2002) The biogenesis and functions of exosomes. *Traffic* 3:321–330
- Tkaczyk C et al (2000) In vitro and in vivo immunostimulatory potential of bone marrow-derived mast cells on B- and T-lymphocyte activation. *J Allergy Clin Immunol* 105:134–142
- van Esch BC, Gros-van Hest M, Westerbeek H, Garssen J (2013) Sensitizing capacity and allergenicity of enzymatically cross-linked sodium caseinate in comparison to sodium caseinate in a mouse model for cow's milk allergy. *Toxicol Lett* 218:50–55
- Volpin G et al (2014) Cytokine levels (IL-4, IL-6, IL-8 and TGFbeta) as potential biomarkers of systemic inflammatory response in trauma patients. *Int Orthop* 38:1303–1309
- Walsh J et al (2016) Differentiating milk allergy (IgE and non-IgE mediated) from lactose intolerance: understanding the underlying mechanisms and presentations. *Br J Gen Pract* 66:e609–611
- Wang J, Sampson HA (2007) Food anaphylaxis. *Clin Exp Allergy* 37:651–660

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