

Honeybee venom secretory phospholipase A₂ induces leukotriene production but not histamine release from human basophils

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Summary

The role of basophils in an anaphylactic response is well recognized but is usually masked by mast cells, which contain similar mediators for the induction of generalized vasodilatation and laryngeal constriction. The rapid onset of systemic anaphylactic symptoms, particularly in insect stings and ingested food, suggest that basophils, a circulating pool of cells containing histamine and other potent mediators such as leukotrienes, may be more involved in systemic anaphylaxis than originally thought. We wished to examine if secretory phospholipase A₂, a systemic allergen found in honey bee venom (HBV-sPLA₂) may activate basophils directly leading to rapid systemic mediator release. Basophils were isolated from human blood and stimulated with increasing concentrations of HBV-sPLA₂. We found that physiological concentrations of HBV-sPLA₂ induce rapid leukotriene C₄ production from purified human basophils within 5 min, while interleukin (IL)-4 expression and production was induced at later time-points. Histamine release was not induced, signifying that HBV-sPLA₂ did not induce generalized degranulation. Surface expression of CD63, CD69 and CD11b were up-regulated following HBV-sPLA₂ treatment. Stimulation of basophils with anti-immunoglobulin E (IgE) following treatment with HBV-sPLA₂ did not induce more leukotriene release. To investigate the mechanism of leukotriene production, 9–12 octadecadiynoic acid, a cyclooxygenase-1 (COX-1) and 15-lipoxygenase inhibitor, was used and this abrogated leukotriene production. These results indicate that HBV-sPLA₂ can directly activate human basophils *in vitro* to induce leukotriene production.

Keywords: allergy, anaphylaxis, lipid mediators, mast cells/basophils

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Introduction

Anaphylaxis is an acute type-1 hypersensitivity reaction characterized by widespread mast cell degranulation and histamine release resulting in acute bronchoconstriction, hypotension, laryngeal constriction, oral oedema and rash [1]. These reactions can arise in seconds or minutes after exposure to the allergen, and can be fatal. Anaphylactic reactions result mainly from systemic allergens, which can include food, drugs, bee/wasp stings or venoms. These dramatic reactions are due to hypersensitivity to the allergen, and also the systemic distribution of these allergens in the circulation. Allergens are most likely to cause anaphylaxis when they are introduced directly into the circulation through ingestion, skin contact and inhalation.

Mast cells and basophils are involved in the anaphylactic reaction and are metachromatic, express high-affinity immunoglobulin E (IgE) receptors, and are sensitive to the cross-linking of these receptors by specific allergens and anti-IgE, leading to the release of preformed (histamine, kallikrein, tryptase) and newly synthesized mediators [prostaglandins, leukotrienes (LT), platelet activating factor (PAF)] known to be important in allergic inflammation [2].

Basophils, albeit rare, are potent circulating leucocytes involved in allergy and asthma. Basophils are activated mainly through the cross-linking of the high-affinity IgE receptor, by anti-IgE or allergen. Other IgE-independent mechanisms of basophil activation exist, including C5a and formyl-met-leu-phe (FMLP), binding to their G-protein linked receptors, and the direct activation of protein kinase C

(PKC) by phorbol myristate acetate (PMA). Cytokines such as interleukin (IL)-3 and nerve growth factor (NGF) have also been reported to have priming effects on basophils. In fact, IL-18 in the presence of IL-3 directly stimulates basophils and mast cells to produce T helper 2 (Th2) cytokines such as IL-4 and IL-13 [3]. Finally, basophil activation can be induced in non-sensitized individuals by a growing list of parasitic antigens, lectins and viral superantigens [4].

A major allergen in honeybee venom (Hymenoptera *Apis mellifera*) is secretory phospholipase A₂ (HBV-sPLA₂), representing ~12% of crude venom [5]. This type III sPLA₂ catalyses the hydrolysis of the sn-2 ester bond in phospholipids, generating free fatty acids such as arachidonic acid (AA), the important substrate in the production of prostaglandins and leukotrienes. A potent neurotoxin, HBV-sPLA₂ is thought to bind to an N-type sPLA₂ receptor [6] and can induce dendritic cell maturation [7]. Interestingly, honeybee venom has been in the limelight as an allergen used in specific immunotherapy and genetic engineering [8].

The cysteinyl leukotrienes LTC₄, LTD₄ and LTE₄ are synthesized rapidly upon activation on conversion of AA by lipoxygenase enzymes and mediate various inflammatory responses such as enhanced mucus secretion, bronchoconstriction and increased vascular permeability [9]. Inhaled LTC₄ and LTD₄ are 1000 times more potent than histamine at inducing prolonged bronchoconstriction in patients.

It is unknown if basophils respond directly to systemic allergens (food allergens, bee/wasp sting) while in the circulation. Machado *et al.* [10] has shown previously that mast cells, but not basophils, respond to HBV-sPLA₂ *in vitro*. It may be possible that circulating basophils activated by these circulating allergens may release histamine into the circulation, resulting in the systemic symptoms of anaphylaxis. Similarly, the use of HBV-sPLA₂ in venom immunotherapy may be detrimental, with direct effects on basophils in the circulation. In our study, we have investigated mediator production in basophils treated with HBV-sPLA₂. Our results demonstrate that human basophils do not release histamine in response to HBV-sPLA₂ but produce leukotrienes very rapidly, which may play a role in the anaphylactic response.

Materials and methods

Materials

The following reagents were purchased: honey bee venom sPLA₂, 9,12-octadecadiynic acid (Cayman Chemical, Ann Arbor, MI, USA), phosphate-buffered saline (PBS), ethylenediamine tetraacetic acid (EDTA) (1st Base, Singapore); and D-(+)-glucose minimum 99.5%, bovine serum albumin (BSA), histamine diHCl, anti-IgE conjugated to fluorescein isothiocyanate (FITC), goat anti-human IgE and o-phthalaldehyde (OPT) were all from Sigma (St. Louis, MD, USA). Leukotriene B₄/C₄/D₄ enzyme-linked immunosorbent assay, Percoll (Amersham, Little Chalfont

UK); IL-4 ELISA (eBioscience, San Diego, CA, USA); CD63 R-phycoerythrin (R-PE)-conjugated mouse anti-human monoclonal antibody (mAb) and CD69 CY-chrome-conjugated mouse anti-human mAb (BD Biosciences, Franklin Lake, NJ, USA), magnetic affinity cell sorting (MACS) basophil isolation kit, magnetic cell separator midi-MACS, MACS column TypeLS⁺/VS⁺, MACS preseparation filter (Miltenyi Biotec, Bergisch Gladbach, Germany).

Basophil purification

Human basophils were purified from either freshly prepared buffy coats (leucocyte concentrate of 450 ml venous blood) obtained from healthy blood donors undergoing routine blood donation or from venipuncture (100 ml) of whole blood from healthy and consenting volunteers, as described previously with slight modifications [10]. Volunteers were not allergic to honey bee venom and did not report to have had previous anaphylactic reactions. This was approved by the National University of Singapore Institutional Review Board. Briefly, venous blood was anti-coagulated with 10 mM EDTA, centrifuged to obtain the buffy coat and diluted with PBS/EDTA supplemented with 0.2% BSA and 0.1% dextrose (PEBD). The purification procedure involved a two-step process consisting of Percoll density centrifugation and negative selection using a MACS basophils isolation kit. The diluted buffy coat was layered over a triple Percoll density gradient (1.085/1.075/1.065) and centrifuged at 500 g for 30 min at room temperature. Basophils collected were predominantly from the interphase of 1.075 g/ml at purities ranging from 1% to 40%. The Percoll-enriched cells were washed twice with PEBD buffer and then purified further by negative selection using the MACS basophil isolation kit essentially according to the manufacturer's protocol. The cells were isolated by negative selection using immunomagnetic removal of contaminating monocytes and lymphocytes with a MACS LS⁺ column (Miltenyi Biotec). Basophil purity ranged between 90 and 100%, as determined from Kimura staining by counting using a Neubauer chamber, with the mean yield of approximately $1-2 \times 10^6/100$ ml of blood and more than 95% viable cells using Trypan blue exclusion. Contaminating cells were exclusively mononuclear leucocytes.

Drug treatments

Purified basophils ($1 \times 10^6/\text{ml}$, 100 μl) were resuspended in either PBS + 1 mM CaCl₂ + 1 mM MgCl₂ for the short-time incubation or IMDM + 10% FBS + 1 mM MgCl₂ + 1 mM CaCl₂ (medium incubation) for the 16 h time-points. Cells were preincubated at 37°C for 5 min before stimulation with HBV-sPLA₂ (final concentration of 0.1 ng/ml – 100 ng/ml, $n = 5-8$). In some experiments, cells were preincubated with a 15-lipoxygenase inhibitor 10 min prior to stimulation ($n = 5$). In the anti-IgE experiments ($n = 5$), cells were either pretreated with HBV-sPLA₂ for 30 min or co-treated with

goat anti-human-IgE (1 : 10 000) for an additional 30 min before supernatants were collected for mediator release. Controls comprised cells incubated with the stimulus in the absence of inhibitors as well as cells incubated in the buffer or media alone.

Flow cytometric analysis

Surface expression of CD63, CD69, CD11b and CD49d were analysed by flow cytometry using fluorescence activated cell sorting (FACS), as described previously [11]. For CD63 and CD69, basophils were double-stained with anti-IgE FITC-conjugated antibody and anti-CD63 PE-conjugated antibody or anti-CD69 PECy5-conjugated antibody for 30 min at 4°C. For CD11b and CD49d, cells were stained first with the 1° antibody, washed and incubated with polyclonal PE-conjugated rabbit anti-mouse IgG and anti-IgE FITC-conjugated antibody for 45 min at 4°C. Flow cytometry was performed using a Becton Dickinson FACSCalibur flow cytometer. Basophils were distinguished on the basis of different forward-scatter and side-scatter properties (R1; Fig. 2), and gates (R2) for anti-IgE-positive cells were set to eliminate contamination. Cell surface expression (PE-stained) of the indicated molecules was identified from the R2 region. The raw mean fluorescence intensity (MFI) of the isotype control sample was subtracted from the raw MFI of the corresponding test sample.

Mediator and cytokine assay

Cell-free supernatants were collected and stored at -30°C until the relevant assays were performed. Histamine release into the supernatant was measured using OPT-dependent non-automated fluorimetry, as described previously [12]. Using this method, total histamine from cells could not be obtained with perchloric acid or Triton-X treatment as this interfered with the fluorimetric analysis. Thus, histamine release is presented as histamine released over spontaneous levels in ng/ml. LTC₄/D₄/E₄ was measured using an acetylcholinesterase competitive enzyme immunoassay kit, following the manufacturer's instructions. IL-4 levels were measured using a ready-SET-go kit following the manufacturer's instructions.

Data analysis

Statistical differences were calculated on original values using one-way analysis of variance (ANOVA). Individual groups were compared using the two-tailed paired Student's *t*-test, comparing data from the same individual. Results are expressed as mean ± standard error of the mean (s.e.m.). A value of *P* < 0.05 was taken as significant.

Results

In our initial experiments, we examined the ability of HBV-sPLA₂ to activate purified basophils directly *in vitro*. Human

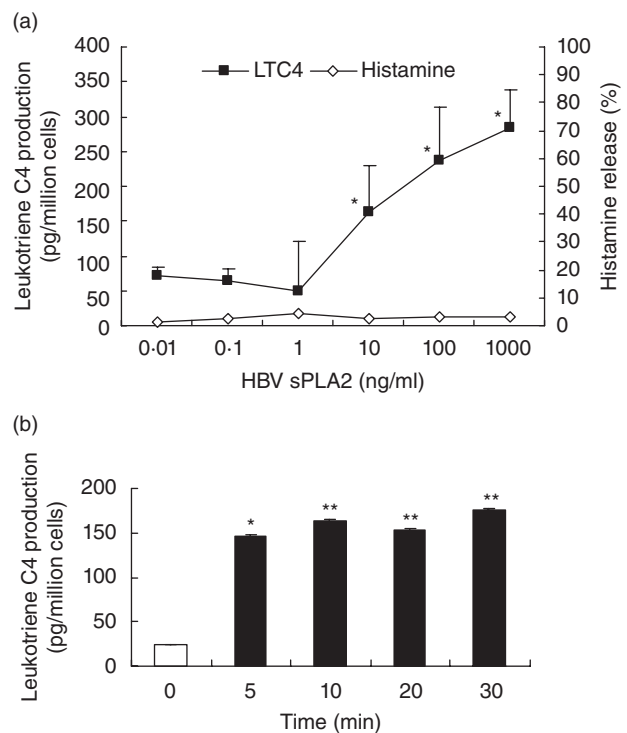
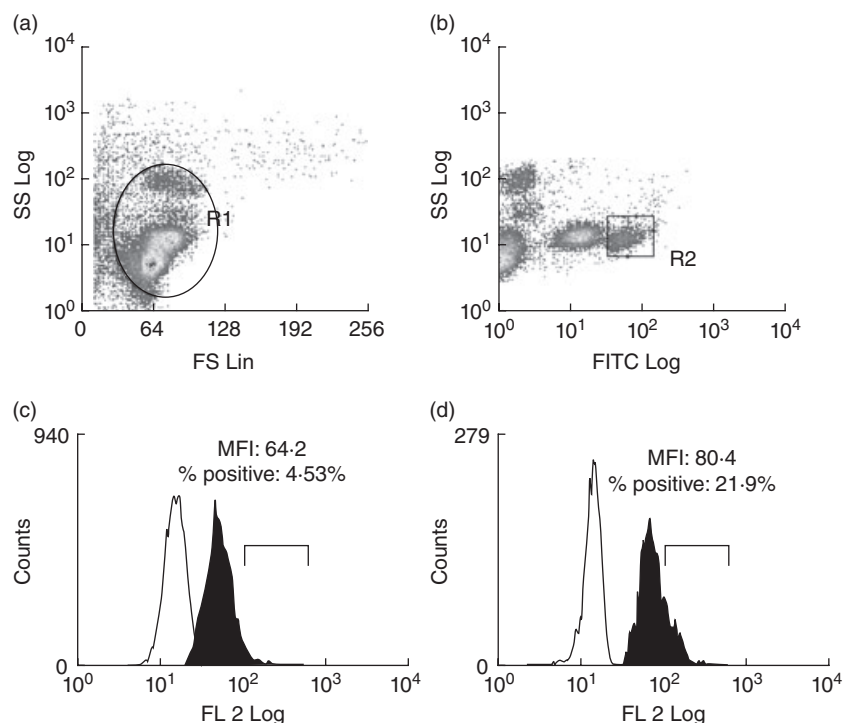


Fig. 1. Honey bee venom secretory phospholipase A₂ (HBV-sPLA₂) directly induces leukotriene production but not histamine release from human basophils. Purified human basophils were treated with (a) increasing concentrations (0.01 ng/ml–1 µg/ml) of HBV-sPLA₂ for 30 min, and (b) 10 ng/ml for increasing times (0–30 min). Histamine concentration in the cell-free supernatants was analysed using o-phthalaldehyde (OPT) fluorescence (see Materials and methods). Spontaneous release was 3% of total histamine content. Leukotriene (LT)C₄/D₄/E₄ concentration in the cell-free supernatants was analysed using a commercially available competitive enzyme-linked immunosorbent assay (ELISA). Basal concentration of LTC₄ was 24.5 ± 1.6 pg/10⁶ cells. Data are presented as mean ± standard error of the mean of *n* = 5–8 experiments. **P* < 0.05; ***P* < 0.01 versus untreated control.

basophils were stimulated with increasing concentrations (0.01 ng/ml–1000 ng/ml) of HBV-sPLA₂ for 30 min. Figure 1a illustrates that no histamine was released following HBV-sPLA₂ treatments at all concentrations. However, HBV-sPLA₂ produced a concentration-dependent response in the production of cysteinyl leukotrienes LTC₄, LTD₄ and LTE₄. The highest production of leukotrienes was seen at the highest concentration added 1 µg/ml (284.3 ± 24.6 pg/million cells). A significant response was seen at 10 ng/ml HBV-sPLA₂ (163.8 ± 14.1 pg/million cells; *P* < 0.05). Thus, subsequent experiments were performed at the suboptimal concentration of 10 ng/ml HBV-sPLA₂.

Next, we performed a time-course of HBV-sPLA₂ on LTC₄ production. Anaphylactic responses can occur within minutes *in vivo*, and thus early time-points were chosen. Treatment of basophils with 10 ng/ml of HBV-sPLA₂ produced time-dependent increases in LTC₄ production

Fig. 2. Basophil identification using side-scatter and fluorescein isothiocyanate (FITC) anti-IgE staining. Semi-pure basophils were double-stained with anti-IgE FITC-conjugated antibody and anti-CD63 phycoerythrin-conjugated antibody and analysed by flow cytometry. (a) Basophils and other peripheral blood mononuclear cells were distinguished on the basis of different forward-scatter and side-scatter properties (R1). (b) Gates (R2) for anti-IgE-positive cells were set to eliminate contamination by lymphocytes and monocytes. Surface expression of CD63 on (c) unactivated and (d) honey bee venom secretory phospholipase A₂ (HBV-sPLA₂)-activated basophils. The clear line histogram illustrates the fluorescence of cells stained with isotype-matched antibody. The data are representative of four independent experiments, showing similar results.



(Fig. 1b). Significant production of LTC₄ was observed by 5 min (146 ± 2.2 pg/million cells; $P < 0.05$). Even a 30-s stimulation of basophils with 10 ng/ml of HBV-sPLA₂ induced a $514 \pm 112\%$ increase in LTC₄ release (data not shown). Furthermore, this concentration of PLA₂ did not induce LTC₄ production from cells obtained from the mononuclear layer, the major contaminants of basophil isolation (data not shown), indicating that basophils were the major producers of LTC₄ in HBV-sPLA₂ stimulation.

We also examined the ability of HBV-sPLA₂ to activate basophils to induce IL-4 production. Treatment of cells with 10 ng/ml HBV-sPLA₂ induced enhancement of IL-4 production at later time-points of 2 h ($109.7 \pm 58.5\%$ increase, $P < 0.1$) and 16 h ($41.6 \pm 13.7\%$ increase, $P < 0.01$) compared to untreated cells (61.3 ± 4.1 pg/million cells). No IL-4 production was seen at 30 min of HBV-sPLA₂ incubations (63.3 ± 7.7 pg/million cells).

The activation status of human basophils was also examined using flow cytometric analysis of activation markers CD63 and CD69, as well as adhesion molecules CD11b and CD49d. Basophils were identified in the lymphocyte population by forward-scatter and side-scatter, and stained positive for the high-affinity IgE receptor (Fig. 2). Basophils treated with 10 ng/ml HBV-sPLA₂ for 30 min exhibited a slight, yet significant increase in cell surface expression of CD63 and CD69 (Fig. 3a). CD11b expression was slightly up-regulated ($15 \pm 4.1\%$, $P = 0.07$), but CD49d expression was not up-regulated significantly with the treatment with HBV-sPLA₂ (Fig. 3b). Treatment with the basophil activator, anti-IgE (at a suboptimal concentration of 1 : 10 000 dilution of the antibody), induced a significant up-regulation of

CD11b ($50.6 \pm 7.9\%$ increase, $P < 0.05$) as well as CD49d ($52.8 \pm 7.6\%$, $P < 0.05$).

Next, we investigated if HBV-sPLA₂ could act as a priming factor for subsequent IgE activation (Fig. 4a). To achieve this, human basophils were treated with 10 ng/ml HBV-sPLA₂ for 30 min prior to stimulation with goat anti-human IgE (1 : 10 000). In this set of experiments, treatment with HBV-sPLA₂ alone induced a $113 \pm 33.4\%$ increase in leukotriene production when compared to the control. Basophils stimulated with anti-IgE alone similarly produced $155.3 \pm 58\%$ more LTC₄ than untreated cells. However, when basophils were pretreated with HBV-sPLA₂ and treated subsequently with anti-IgE, no further increase in LTC₄ release was observed when compared to anti-IgE treatment alone. Similarly, when basophils were treated simultaneously with HBV-sPLA₂ and anti-IgE for 30 min, LTC₄ production was not significantly different from anti-IgE alone, but was increased significantly when compared to HBV-sPLA₂ alone (data not shown). This indicates that anti-IgE can further activate basophils after stimulation with HBV-sPLA₂ but HBV-sPLA₂ could not prime basophils to release more mediators to anti-IgE stimulation.

Finally, we investigated the possible mechanism of action of HBV-sPLA₂. Basophils were pretreated with 9,12-octadecadiyniic acid or Ro3-1314 (OCA), a cyclooxygenase-1 (COX-1) and 15-lipoxygenase inhibitor (15-LO). Addition of OCA 15 min prior to HBV-sPLA₂ treatment completely abolished LTC₄ production induced by sPLA₂ by 98% (Fig. 4b). This indicates that either COX-1 or 15-LO is involved intricately in the basophil activation induced by HBV-sPLA₂.

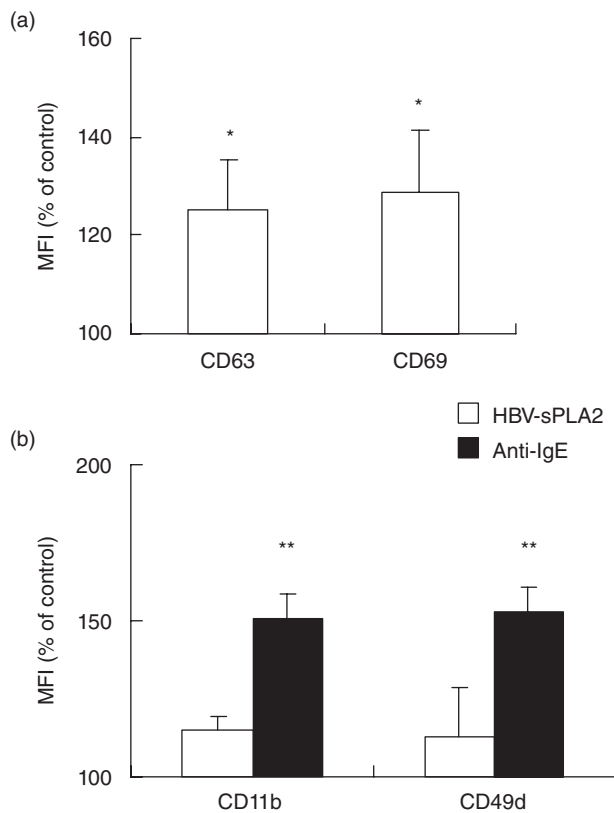


Fig. 3. Honey bee venom secretory phospholipase A₂ (HBV-sPLA₂) treatment of human basophils increases CD63, CD69 and CD11b expression. Purified human basophils were treated with either 10 ng/ml of HBV-sPLA₂ or anti-IgE (1 : 10 000 dilution) for 30 min. Surface expression of (a) CD63, CD69 and (b) CD11b and CD49d were analysed using flow cytometry. Results are presented as percentage change of mean fluorescence intensity over the untreated control $\pm$ standard error of the mean of $n = 5$ experiments. * $P < 0.05$ versus untreated control.

Discussion

This study demonstrates the ability of HBV-sPLA₂, a potent enzyme and allergen in honeybee venom anaphylaxis, on the activation and production of lipid mediators from human basophils. However, basophil degranulation was not induced, as no histamine release was observed.

The rapid onset of symptoms of systemic anaphylaxis after a bee sting (< 5 min) is associated with circulating histamine release, i.e. widespread vasodilatation and laryngeal constriction and hives, usually thought to be induced by stimulation of mast cells and basophils by specific IgE [1]. The contribution of basophils in the anaphylactic response is recognized but is somewhat neglected, due to the low numbers of basophils in the circulation. As they contain histamine and express the high-affinity IgE receptor, their functions are always thought to be similar to mast cells, central effector cells in the development of IgE-dependent anaphylaxis [2]. Previous studies have described anaphylactic responses in mast cell-deficient mice [13], implying that

other cells may release mediators to induce systemic anaphylaxis. Similarly, the hypotensive response observed in rats induced by antigen-mediated anaphylaxis is independent of mast cell activation [14]. Basophils containing vast amounts of preformed histamine, and capable of producing high levels of leukotrienes and cytokines, may definitely be a candidate.

Skin test reactions to honeybee venom and venom-specific IgE indicate that honeybee venom contains allergens. Interestingly, 20% of people in the general population with no previous insect sting history showed a positive test [15], suggesting a non-IgE-mediated response. The major allergen of honeybee venom is secretory PLA₂, and has been shown to modulate the allergic response through IgE-mediated mechanisms in basophils and mast cells [16]. However, it is unclear if it can induce basophil activation directly in the absence of IgE sensitization. We have shown in this study that HBV-sPLA₂ is not able to induce significant histamine release at low concentrations. This is in accor-

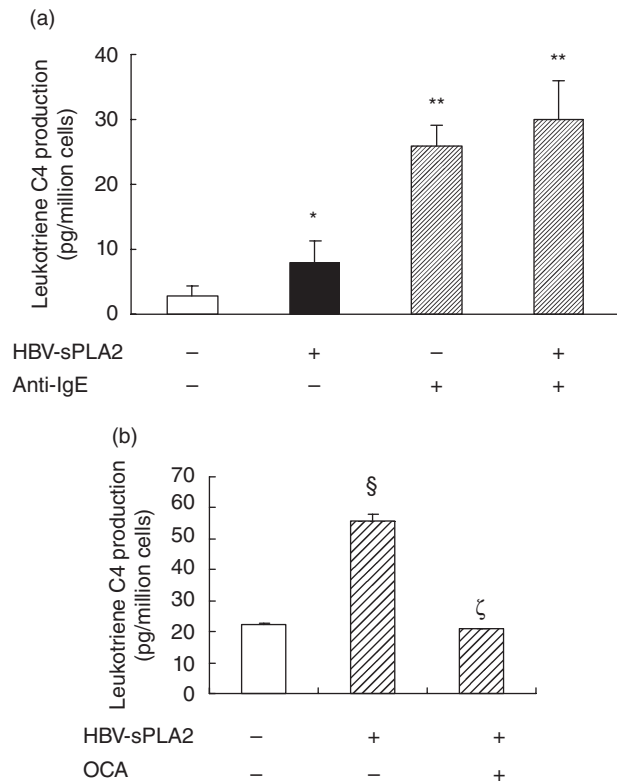


Fig. 4. Possible mechanisms of honey bee venom secretory phospholipase A₂ (HBV-sPLA₂). (a) Purified human basophils were treated with 10 ng/ml of HBV-sPLA₂ for 30 min prior to stimulation with anti-IgE (1 : 10 000) for 30 min. (b) Purified human basophils were preincubated with 10 μ M of 9,12-octadecadiynic acid 15 min prior to treatment with 10 ng/ml of HBV-sPLA₂ for 30 min. Mediator production was analysed in cell-free supernatants as described in Fig. 1. Data are presented as mean $\pm$ standard error of the mean of $n = 5$ experiments. ** $P < 0.01$, * $P < 0.05$, § $P < 0.1$ versus untreated control; ζ $P < 0.05$ versus HBV-sPLA₂-treated cells.

dance with a previous study where HBV-sPLA₂ did not induce histamine release in basophils, but induced IgE-independent histamine release and IL-4 production in RBL-2H3 cells and human lung and human skin mast cells [10].

We took a step further to study the effect of HBV-sPLA₂ on leukotriene production. HBV-sPLA₂ induced a rapid concentration-dependent increase in LTC₄ production, with evident production after 5 min of treatment. The rapid production of LTC₄ is somewhat surprising, but as PLA₂ is an enzyme involved in the AA pathway, treatment of cells with exogenous PLA₂ may lead to the formation of LTC₄ and other eicosanoids. However, Hundley *et al.* [17] showed previously that exposure of recombinant sPLA₂ to basophils increased free AA levels with no effect on LTC₄ release. It is possible that the introduction of HBV-sPLA₂ into the cellular milieu increases the amount of free AA, which can also increase the production of LTC₄ by increasing the activity of 5-lipoxygenase (LO). Similarly, a recent report demonstrated that HBV-sPLA₂ induces dendritic cell activation and maturation by increasing AA release and prostaglandin E₂ (PGE₂)/PAF production [7]. This may explain the activation of basophils seen in our study. Other sPLA₂ groups, e.g. group V sPLA₂ and group X sPLA₂, can act upon neighbouring eosinophils, increasing AA release and LTC₄ production [18] and inducing COX-2 activity and subsequent PGE₂ production in macrophages [19]. The structure of HBV-sPLA₂ is very similar to mammalian sPLA₂, which may explain the potent activities of HBV-sPLA₂ in cells.

Basophils, especially those of bee venom-allergic patients [20], have been shown to bind to HBV-sPLA₂. This suggests that basophils may have receptors for HBV-sPLA₂ which, when activated, can induce basophil activation and histamine release, but to date the expression of HBV-sPLA₂ receptors on basophils or mast cells has not been reported. Indeed, HBV-sPLA₂ binds to N-type PLA₂ receptors in the brain, linking to its neurotoxic effects [6]. Mutants of HBV-sPLA₂ with low affinity to the N-type receptor do not possess neurotoxic properties, indicating the importance of this particular receptor [6]. Further confirmation that HBV-sPLA₂ may have direct effect on basophils comes from studies showing that the enhancement of mast cell histamine release and degranulation by HBV-sPLA₂ and other potent allergens such as house dust mite was critically dependent on enzymatic activity rather than IgE [21]. We can be certain that our basophil donors were healthy, and not sensitized to HBV-sPLA₂ (from donor consent and survey), thus the observed effect of HBV-sPLA₂ on basophil mediator release is not IgE-mediated. However, it is interesting to note that while basophils from healthy donors release mediators after incubation with HBV-sPLA₂, not all subjects will develop anaphylaxis after a honey bee sting. This is due probably to the fact that *in vivo* basophil activation is governed by environmental factors, and experiments performed on isolated basophils *in vitro* do not reflect physiological conditions fully. It will be important to demonstrate the importance of

basophil activation during anaphylaxis *in vivo*. It is also possible that the symptoms of anaphylaxis may involve the release of histamine into the circulation, synergizing with the effects of the leukotrienes released directly upon stimulation with the enzyme.

Our results also indicate that HBV-sPLA₂ induces the expression and production of the Th2 cytokine, IL-4. The increase in IL-4 production is observed within 2 h of stimulation with HBV-sPLA₂. IL-4 is a major cytokine driving the Th2 immune response and the induced production by HBV-sPLA₂ may prove to have importance in the allergic response. IL-4 together with CD40L can induce B cell class-switching from IgM to IgE [22]. According to the study by Machado *et al.* [10], the direct stimulation of IL-4 production by enzymatic allergens such as HBV-sPLA₂ is of particular significance, as substances modulating IL-4 secretion may act as IgE-specific adjuvants and induce an IgE response to other antigens. Similarly, in IL-4 knock-out mice IgE production is totally abolished [23]. IL-4 can also attract circulating eosinophils and basophils by inducing specific adhesion molecule expression, i.e. vascular cell adhesion molecule (VCAM)-1 on endothelial cells [24]. Interestingly, IL-4 can prime mast cells, increasing sensitivity to other mediators such as substance P [25] as well as inducing mediator release in synergy with stem cell factor [26] and IgE [27]. All these point towards the importance of IL-4 production during an anaphylactic response.

Preincubation with HBV-sPLA₂ prior to the addition of anti-IgE did not induce more mediator release. This indicates that HBV-sPLA₂ does not prime basophils like other priming factors, IL-3 or NGF [28], and may activate a separate signalling pathway leading to rapid basophil degranulation and activation. Group IA and IIA sPLA₂ activates and phosphorylates p38 and ERK1/2 MAPK for mediator production [29], while group IB sPLA₂ activates neutrophils to produce IL-8 via ERK and nuclear factor (NF)-κB [30]. Similarly, group IB sPLA₂ stimulates LTB₄ production from human neutrophils without affecting arachidonic acid release [31]. It may be possible that HBV-sPLA₂ works in a similar fashion in our study, but further work needs to be performed to determine the mechanisms through which HBV-sPLA₂ exerts its function. We hypothesized that HBV-sPLA₂ could be regulating basophil mediator release by the production of 15-LO. To address this hypothesis further, we treated the cells with a 15-LO inhibitor, OCA, which completely abrogated leukotriene production. However, OCA is more potently a COX-1 inhibitor, inhibiting 95% and 68% of COX-1 and 15-LO enzymatic activities, respectively, when used at a concentration of 48 μM. At the 10 μM concentration used in our study, it is more likely that COX-1 may be more involved in the basophil activation induced by sPLA₂ rather than 15-LO, but it does not rule out that LTC₄ production may be an enzymatic process.

In conclusion, we have shown that allergens which induce anaphylaxis, such as HBV-sPLA₂, can induce rapid basophil

leukotriene production within 5 min through a 15-LO/COX-1-dependent manner. This demonstrates that anaphylaxis can occur through the direct activation of basophils to induce and perpetuate the anaphylactic response. This could account for the positive skin test reactions observed in patients with no previous history of insect stings.

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