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Prostaglandin E2 Decreases Basophil Activation In Patients With Food-Induced Anaphylaxis

Short Title: Eicosanoid metabolism may play a role in food anaphylaxis

Key words: anaphylaxis, basophils, eicosanoids, food allergy, PGE₂

To the Editor:

Adult-onset food allergy may manifest as anaphylaxis in up to half of patients in some series¹. Mast cells and basophils are known to mediate immunoglobulin E(IgE)-dependent anaphylaxis. Food-induced anaphylaxis(FAn) is only partially determined by the level of allergen sensitization and can occur with low specific IgE². This finding suggests that anaphylaxis depends, at least in part, on immunomodulators that regulate the intensity of the reactions². However, very little is known on the role of immunomodulators in the prevention of FAn. Mast cells/basophils can be a source of autocrine regulators to suppress excessive cell degranulation, such as prostaglandin E₂(PGE₂)². Previous observations showed that misoprostol(PGE₁ agonist), inhibits mast cell/basophil activation and proved to be clinically effective³. Observations in humans, animal models and *in vitro* studies have demonstrated that PGE₂ abrogates allergen-induced mast cell activation^{2,4,5}. However, it has also been shown that PGE₂ may have opposite effects,

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exacerbating or inhibiting antigen-mediated reactions depending on the ratio of the four PGE₂ receptors (EP1-EP4) expressed^{6,7}.

We hypothesize that a defective PGE₂ metabolism is involved in the development of FAn and that exogenously administered PGE₂ can stabilize allergen-induced basophil activation(BA). Thus, we aimed to test in patients with FAn related to peach lipid transfer protein (Pru p 3; LTP) whether: (1)exogenously administered PGE₂ inhibits IgE-dependent basophil activation; (2)the expression profile of PGE₂ receptors on basophils is altered and (3)PGE₂ baseline plasma levels are altered as well.

10 healthy volunteers(HV) and 25 patients were recruited based on a clinical history of anaphylaxis elicited by peach and sensitized to Pru p 3 (demographic data-Suppl.Table 1). PGE₂ plasma levels were measured by ELISA. EP receptors gene expression was measured in isolated basophils by quantitative real-time PCR. An *in vitro* BA test(BAT) measured as expression of CD63, was performed with Pru p 3, anti-IgE high affinity receptor(FcεRI), N-Formylmethionine-leucyl-phenylalanine (fMLP;2μM) and PGE₂ alone and in combination. *Detailed Methods- Online Repository*.

No differences in PGE₂ levels were observed(**Suppl.Fig.1**). No EP1 (mRNA) expression was found in basophils from any group, and EP2 expression was similar. EP3 was significantly increased and EP4 decreased in FAn(**Fig.1a**). The ratio between receptors with known pro-inflammatory(EP3) and anti-inflammatory(EP2-EP4) activity was also significantly higher in FAn(**Fig.1b**)

PGE₂ inhibited Pru p 3 BA at all concentrations (only statistically significant at 10⁻⁴M). PGE₂ significantly decreased Pru p 3, anti-FcεRI and fMLP-mediated BA in FAn(**Fig.2a**). In HV, PGE₂ significantly inhibited both anti-FcεRI- and fMLP-mediated activation(**Fig.2b**).

Exploring the suppressive effects of PGE₂ on BA may uncover unknown pathogenic mechanisms. In this study, we have demonstrated that (1)PGE₂ modifies IgE-mediated basophil reactivity, (2)FAn patients have a particular mRNA EP expression pattern in basophils that may favor its activation by endogenous PGE₂ and (3)there are no differences in PGE₂ plasma concentration.

We did not find differences in baseline PGE₂ in FAn compared with HV. Despite the relatively small sample, this observation suggests that the constitutive production of PGE₂ is not affected in FAn. There are almost no studies regarding PGE₂ values in anaphylaxis. Recently, Rastogi et al.⁸ found that hymenoptera anaphylaxis patients had lower baseline PGE₂ serum levels than HV (142.3±14.1 and 324.7±33.7 pg/mL, respectively). Those values are much higher than the observed in our patients at baseline (51.6±3.7 pg/mL), and closer to the values after 60 minutes at room temperature(RT) (365.3

pg/mL). We have shown, as others before⁹, that PGE₂ metabolism is activated by simply leaving the sample at RT. Therefore, considering that there is no information regarding the specifics of sample collection in Rastogi's study, the differences observed may be considered with caution. The differences in the etiology (hymenoptera vs food) and samples (serum vs plasma) may also account for the discrepancies between these studies.

We found that FAn patients have a higher EP3 expression(mRNA) in basophils, known as a pro-inflammatory receptor, and lower expression of anti-inflammatory EP4, as well as higher EP3/EP2+EP4 ratio. It has been suggested that the expression pattern of EP receptors in a particular tissue/cell type may define PGE₂ final effect^{5,6}. In this line, it has been shown that PGE₂ activates and inhibits human mast cells via EP3 and EP2/EP4 signalling pathway, respectively^{6,7}. Therefore, the balance favoring destabilizer(EP3) vs stabilizers(EP2+EP4) receptors might predispose basophils from FAn to be activated by endogenous PGE₂, as previously demonstrated in mast cells^{6,7}.

The study also shows that PGE₂ decreases BA through both the IgE/FcεRI and fMLP receptor in all groups. This finding is in keeping with previous observation showing that PGE₂ can regulate mast cell activation, suggesting its potential role in suppressing IgE-mediated reactions^{2,5,8} but also opens new perspectives on modulation of other activation pathways. Although there is no evidence of PGE₂ protective effects in food allergy/anaphylaxis models, misoprostol has indeed shown to suppress symptoms in wheat-dependent exercise-induced anaphylaxis and IgE-mediated histamine release^{3,4}.

A possible explanation of these apparently conflicting results is that we investigated the role of PGE₂ in BA in two different scenarios: endogenous production and exogenous administration with a pharmacological/therapeutic purpose. Although the concentration reached endogenously during an anaphylaxis is not known, it is conceivable that it is much lower than the PGE₂ concentration used in our study. Moreover, the activation of EP receptors is dependent on both PGE₂ concentration and the receptor². The concentration of PGE₂ used in our experiments may be high enough to bind all EP receptors regardless of its affinity, resulting in a final inhibition. However, one of our objectives was explaining the mechanism of action of PGE₂ in preventing BA. Therefore, the used concentrations cannot be considered as a reference for human use.

Further studies are needed to address some limitations. No differences in PGE₂ baseline levels were observed; nevertheless, exploring the amount of PGE₂ released during IgE-induced reactions in patients with different clinical severity would be of interest.

In summary, we first reported the differential mRNA expression of PGE₂ receptors in basophils of food anaphylaxis patients. We also demonstrate, despite the possible limitations in the design of BAT tests, the efficacy of PGE₂ to abrogate IgE-dependent basophil activation, a result which supports further exploring the role of the eicosanoid metabolism in food anaphylaxis.

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The authors declare that they have no conflict of interest.

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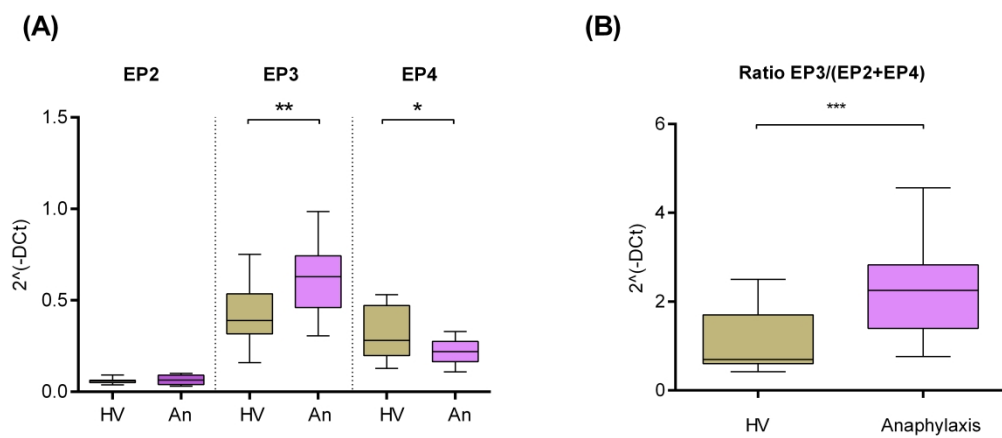
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Figures legends

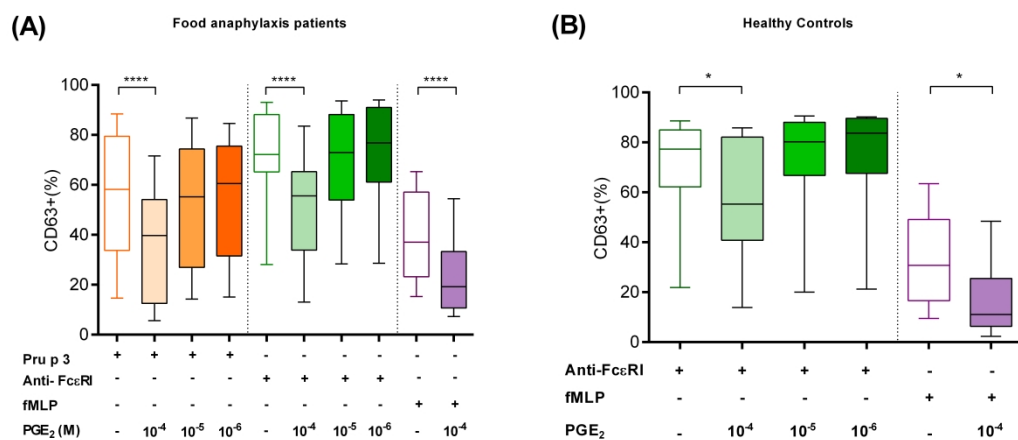
Figure 1. Box plot and whiskers of PGE₂ receptors mRNA expression in isolated basophils in healthy volunteers (HV) vs food-anaphylaxis patients (FAn). (A) Expression profile of PGE₂ receptors (EP); (B) Ratio EP3 (pro-inflammatory)/EP2+EP4 (anti-inflammatory). A t-test was used to compared HV vs An. *P<0.05; **P<0.01; ***P<0.001.

Figure 2. Box plot and whiskers of median basophil activation (%CD63+) in anaphylaxis patients, using (A) Pru p 3, anti-FcεRI and fMLP in food-anaphylaxis patients, and (B) anti-FcεRI and fMLP in healthy controls, both combined with PGE₂. A paired t-test was used to compare the effect of PGE₂ on basophil activation. Asterisks represent significance (*P<0.05; ****P<0.0001) compared to Pru p 3/Anti-FcεRI/fMLP activation. Pru p 3=peach lipid transfer protein; PGE₂=prostaglandin E₂; FcεRI: IgE high affinity receptor; fMLP: N-Formylmethionine-leucyl-phenylalanin .

Fig. 1

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Fig 2



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Prostaglandin E2 Decreases Basophil Activation In Patients With Food-Induced Anaphylaxis

Supplementary data

Material and methods

Patient selection

Patients were recruited based on a clinical history of anaphylaxis¹ elicited by peach. All patients had a positive skin prick test (SPT) to peach and peach LTP (Pru p 3)-specific serum IgE (sIgE) (ImmunoCAP®, Thermofisher Scientific, Uppsala, Sweden). Specific IgE > 0.10 kU_A/L was considered positive. Total IgE (tIgE) was determined using ImmunoCAP® (Thermofisher Scientific).

Pru p 3 allergic patients were divided into two clinical phenotypes: (1) patients with peach-dependent NSAID-induced anaphylaxis (FDNIA), (2) patients with peach anaphylaxis without the involvement of NSAID. These phenotypes were defined only for the purpose of selecting the Pru p 3 doses to be tested in BAT assays and to rule out NSAID hypersensitivity when needed. Besides that, BAT data from these phenotypes were analyzed together because *in vitro* both groups show the same behavior in response to allergens and NSAIDs regardless of the distinct clinical presentation *in vivo*, as we previously reported² and hereby confirmed with this series. Briefly, we observed that, although NSAID was not necessary to induce anaphylaxis in one of the phenotypes, L-ASA also induced an enhancement of the basophil activation caused by the allergen *in vitro* (similar to FDNIA patients). For confirmation, BAT data from these phenotypes is shown separately in this repository.

Patients with a clinical history of exercise-induced anaphylaxis and NSAIDs hypersensitivity were excluded. Healthy volunteers (HV) with no clinical history of food allergy or NSAID hypersensitivity, and not sensitized to any food or Pru p 3 were recruited.

Informed consent was obtained from all participating subjects. The study was approved by the local ethics committee of the Hospital Clinic (Barcelona, Spain).

NSAIDs oral challenges

NSAID hypersensitivity was ruled out in all patients. Oral challenge with the NSAID involved in the reaction was performed in all patients with FDNIA, according to the EAACI position paper³. Oral challenge with aspirin (500 mg) was performed in all cofactor-independent food anaphylaxis patients, although NSAIDs were not involved in their reactions. Due to the severity of the reactions, FDNIA patients were not challenged with the combination of peach and NSAID, and cofactor-independent food anaphylaxis patients were not challenged with peach.

Basophil activation test (BAT)

Blood samples were collected after assuring that patients had not consumed peach or an NSAID in the last 48 hours. A basophil activation test, measured as expression of CD63 (Flow²CAST®; Bülmann Laboratories AG®, Schönenbuch, Switzerland), was performed right away following the manufacturer's procedure. Stimulation was performed with anti-FcεRI following manufacturer's instructions, fMLP at 2 μM and Pru p 3⁴ at 1.12 ng/mL and 0.013 ng/mL for FDNIA and NSAID-

independent FAn respectively, based on the concentration eliciting 50% of the maximal response (EC50) observed in our previous work ². Prior to stimulation, PGE₂ (10⁻⁴, 10⁻⁵ and 10⁻⁶M; Cayman Chemical (Item № 14010)) was added for 15 minutes at room temperature ⁵.

Flow cytometric analysis

Flow cytometric analysis was performed using a FACS-Canto II flow cytometer (Biosciences, Heidelberg, Germany) and using FACS-Diva[®] software for analysis. Basophils were selected out of the lymphocyte population as SSC^{low}CCR3⁺. At least 500 basophils were assessed to consider the test reliable. Basophil activation was assessed with the percentage of CD63⁺ cells within the total identified basophils. A high percentage of CD63⁺ cells in the negative control tubes (>5% following the manufacturer's recommendations) or a lack of stimulation when using the monoclonal anti-FcεRI antibody (SC, stimulation control), were considered as criteria for excluding the patient from the study. For Pru p 3, an increase of ≥15% of CD63⁺ cells compared with background was considered positive following manufacturer's recommendations for food allergens. Results were expressed as stimulation index (SI), using net percentages of CD63⁺ basophils that were calculated by subtraction of the spontaneous expression from the allergen-induced CD63 expression, as recommended elsewhere⁶.

PGE2 plasma levels

Venous blood samples were collected in polypropylene test tubes coated with EDTA. PGE2 plasma levels were measured using a commercial ELISA kit PGE2-Monoclonal (Cayman chemical, Michigan, USA), following the manufacturer's instructions. It has been demonstrated that PGE2 metabolism is activated over the time in nasal polyps tissues from aspirin-intolerant asthmatic patients left at room temperature for 60 minutes; PGE2 metabolism was not modified over time in nasal mucosa from healthy controls⁷. Thus, we decided to investigate whether there is a differential kinetics of PGE2 by measuring it at baseline (t0) and 60 minutes at RT after drawing (t60). Further PGE2 ex-vivo synthesis was blocked using sterile indomethacin at 5 μM, and samples were immediately centrifuged and stored at -80°C to avoid PGE2 degradation. Venous blood samples were collected in polypropylene test tubes coated with EDTA. PGE2 plasma levels were measured using a commercial ELISA kit PGE2.

Basophil isolation and real-time PCR analysis

Basophils were isolated from whole blood using the EasySep[®] Basophil Isolation Kit (Stemcell Technologies, Vancouver, Canada) following the manufacturer's instructions. PGE2 receptors (EP) and cyclooxygenase (COX) 1 and 2 expression in isolated basophils was evaluated using RT-qPCR. Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Ontario, Canada) according to the manufacturer's protocol. Then 1 μg of RNA was reverse transcribed to cDNA by using a High-Capacity cDNA Reverse Transcription Kit with RNase inhibitor (Applied Biosystems, Life Technologies, California, USA). cDNA amplification was accomplished with a real-time PCR system (7900 HT Fast Real-Time PCR System, Applied Biosystems), TaqMan Fast Universal PCR Master Mix, and TaqMan Gene Expression Assay (Thermofisher Scientific, Uppsala, Sweden) for *PTGS1* Hs00377726, *PTGS2* Hs00153133, *PTGR1-4* (Hs00909194, Hs00168754, Hs00168755, Hs00168761) and *GAPDH* Hs02758991_g1. Target mRNA expression was normalized to GAPDH and was calculated using the ΔΔCt method.

Statistical analysis

Quantitative variables were described by median and interquartile range (IQR) and qualitative variables by absolute frequencies and percentages. Non parametric tests (Wilcoxon for 2 groups and Kruskal-Wallis for more than 2 groups) were used for the statistical analysis. A P value below 0.05 was considered significant. The GraphPad Prism 4.0c, GraphPad Software, Inc, CA, USA, was used.

Results

A total of 33 Pru p 3-allergic patients who had developed anaphylaxis after eating peach were recruited. 56% were female with a median age (interquartile range, IQR) of 40 (34-50). 10 healthy volunteers (6 female) with a median age (IQ) of 38 (32-48) were also recruited. Total IgE, Pru p 3 sIgE and serum tryptase median values (IQR) were: 96.6(45-165) kU/L, 9.7 (2.3-16.3) kU/L and 4.5 (3.5-6.4) ng/mL respectively. Individual values are shown in Suppl. table 1. No other food allergen sensitization other than LTP was detected when screened by microarray ImmunoCAP ISAC® (ThermoFisher Scientific, Uppsala, Sweden) in these patients (data not shown). 8 patients were excluded from the study: a lack of stimulation using the monoclonal anti-FcεRI antibody (SC, stimulation control) was observed in 6 patients, and in 2 patients an increased stimulation with the negative control was found.

PGE2 plasma levels, Basophil isolation/activation and EP receptors mRNA expression

No differences were observed among anaphylaxis patients (FDNIA vs food anaphylaxis) in any of the variables of the study (BAT-Suppl Fig.2; PGE2 levels-Suppl Fig.3; EP mRNA expression-Suppl Fig.4). However, when comparing each of the anaphylaxis groups with the HV, we observed the same results shown when comparing all anaphylaxis patients together with HV.

Suppl Table 1. Demographic characteristics of patients with anaphylaxis

Patient	Age	Gender	Total IgE (kUA/L)	IgE anti-Pru p 3 (kU/L)	NSAID involved in the reaction	Baseline serum tryptase (ng/mL)
1	49	F	150	16.3	Ibuprofen, ASA	3.5
2	49	F	179	38.6	ASA	1.9
3	56	F	16.3	0.9	Ibuprofen	5.4
4	29	F	165	83.1	Ibuprofen	2.9
5	32	F	42.1	3.6	Ibuprofen	9.4
6	36	M	62.5	2.3	ASA, dipyrone	6.4
7	35	M	174	3.2	Ibuprofen	8.9
8	63	M	185	0.9	Ibuprofen	4.9
9	36	F	45	14.2	Dexketoprofen	7.7
10	31	F	45.5	13	--	2.4
11	40	F	228	2.1	-	3.3
12	55	F	104	0.2	-	4.1
13	51	F	207	1.4	-	2.8
14	51	M	508	62.9	-	3.8
15	45	M	974	2.3	-	6.1
16	44	M	67	14.9	-	2.9

17	40	M	19.4	2.2	-	3.2
18	40	M	95	15.7	-	8.2
19	29	M	96.6	3.4	-	5.5
20	33	M	60	0.6	-	2.3
21	28	F	326	24.6	-	4.4
22	42	F	16.3	14.3	-	6.1
23	54	M	139	9.7	-	4.5
24	49	F	277	0.7	-	4.1
25	44	F	39.8	5.9	-	4

F: female, M: male

Grey shadow identifies peach-dependent NSAID-induced anaphylaxis patients.

Figures legends

Suppl Fig 1. Box plot and whiskers of PGE₂ plasma levels in healthy volunteers (HV) and anaphylaxis patients. A paired t-test was used to compare t0 (baseline) vs t60 (60 minutes at RT); ***P<0.001 ****P<0.0001). No differences were observed between groups at t0 and T60.

Suppl Fig 2. Box plot and whiskers of basophil activation (%CD63+) using Pru p 3, anti-FcεRI, fMLP and PGE₂ as a stimulus in (A) FDNIA group and (B) NSAID-independent FA group. A paired t-test was used to compare the effect of PGE₂ on basophil activation. Asterisks represent significance (*P<0.05; **P<0.01 ***P<0.001; ****P<0.0001) compared to Pru p/Anti-FcεRI/fMLP activation. Pru p 3=peach lipid transfer protein; PGE₂=prostaglandin E₂; FDNIA: food-dependent NSAID-induced anaphylaxis; FAn: food anaphylaxis; FcεRI: IgE high affinity receptor; fMLP: N-Formylmethionine-leucyl-phenylalanine.

Suppl Fig 3. Box plot and whiskers of PGE₂ plasma levels in healthy volunteers (HV), food-dependent NSAID-induced anaphylaxis (FDNIA) patients and NSAID-independent food anaphylaxis (FAn-NoNSAID). A paired t-test was used to compare t0 (baseline) vs t60 (60 minutes at RT); ***P<0.001 ****P<0.0001). No differences were observed between groups at t0 and T60.

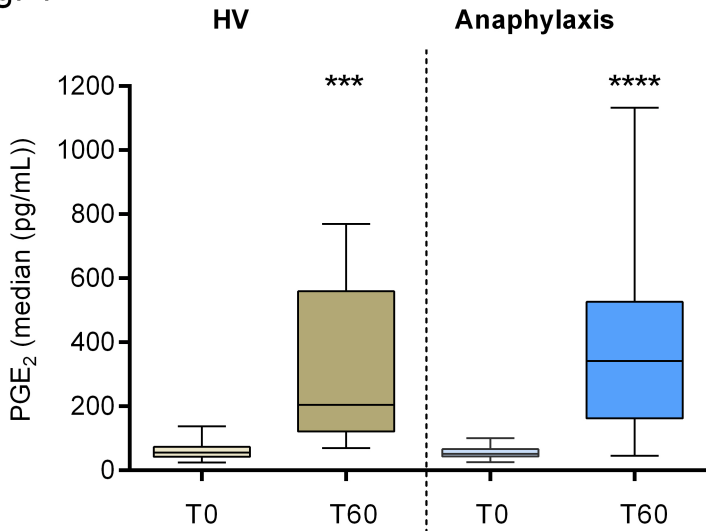
Suppl Fig 4. Box plot and whiskers of PGE₂ receptors expression (mRNA) in isolated basophils in healthy volunteers (HV), food-dependent NSAID-induced anaphylaxis (FDNIA) patients and NSAID-independent food anaphylaxis (FAn-NoNSAID). (A) Expression profile of PGE₂ receptors (EP); (B) Ratio EP3 (pro-inflammatory)/EP2+EP4 (anti-inflammatory). A t-test was used to compare HV vs anaphylaxis groups. *P<0.05 **p<0.01 ***p<0.001.

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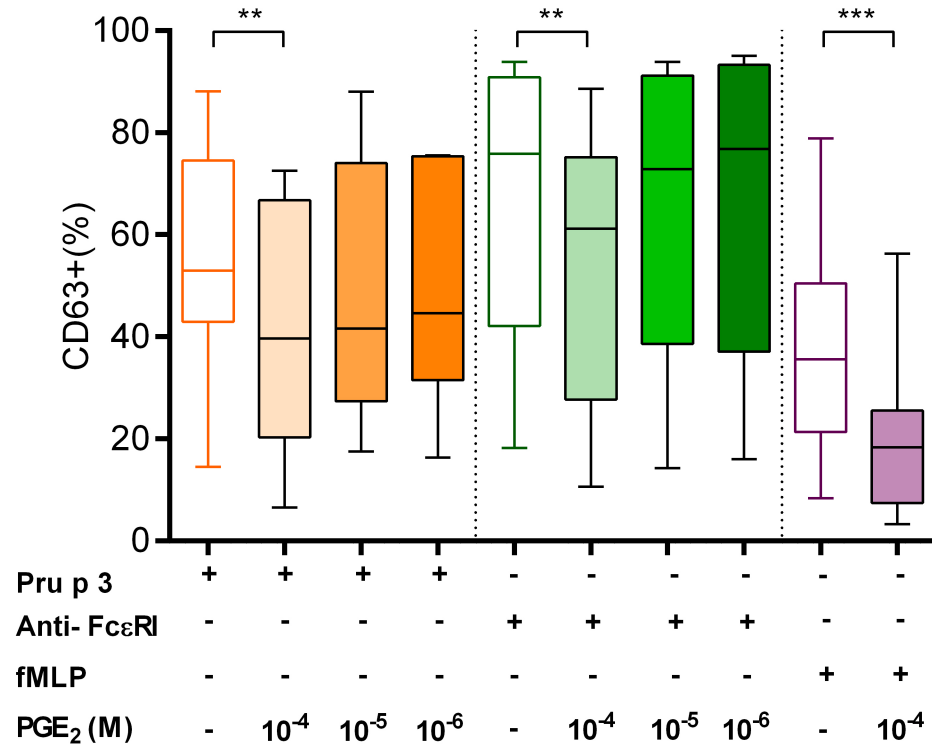
Suppl. Fig. 1



Suppl Fig 2

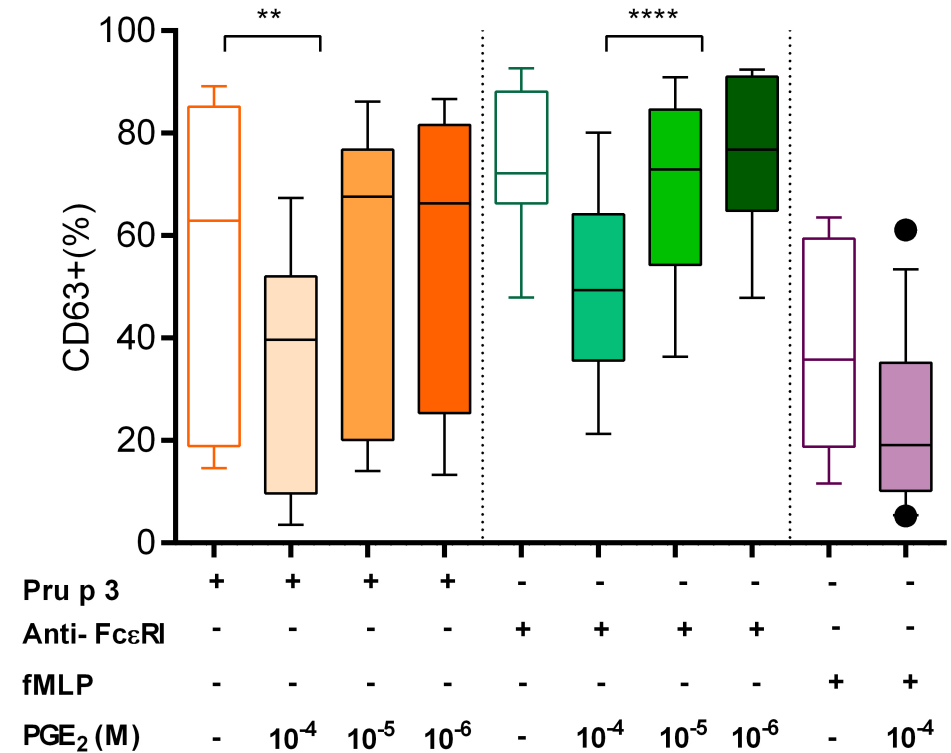
(A)

Food-dependent NSAID-induced anaphylaxis

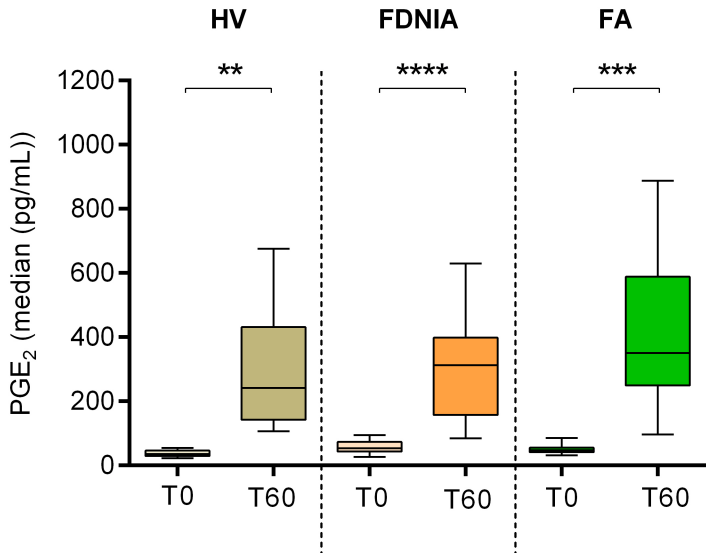


(B)

NSAID-independent food anaphylaxis

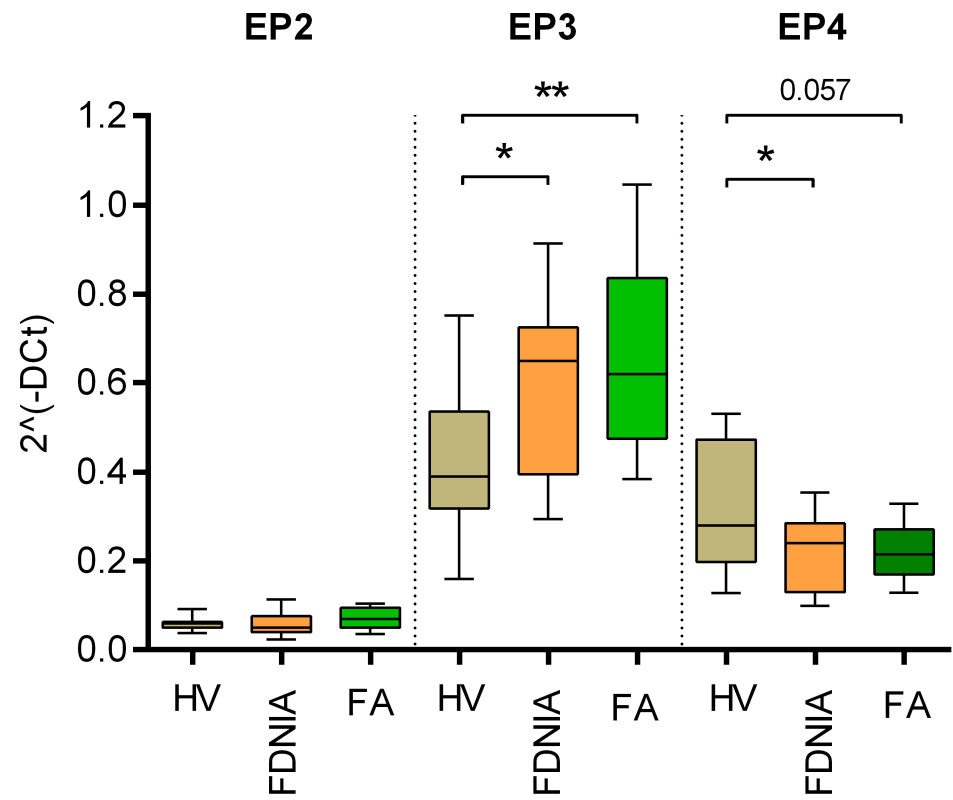


Suppl. Fig. 3



Suppl. Fig. 4

(A)



(B)

