IgE-independent activation of mast cells by perfluoroalkyl compounds

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Introduction

Perfluoroalkyl compounds (PFCs) have been extensively utilized in industrial and consumer products such as surfactants, packaging products, carpet treatment and fabrics. Due to their extreme persistent to degradation, PFCs are accumulated in environment, wildlife and human [1]. In fact, PFCs have been detected in serum from general population as well as in umbilical cord and breast milk [2-4]. As environmental pollutants, PFCs have been suggested to have diverse biological toxicities. Recent animal and human studies have shown that PFCs have immunotoxic effects [5,6]. It has been reported that PFCs exposure reduced immune responses to childhood vaccination with rubella and booster vaccination with diphtheria and tetanus in adults [7,8]. In addition, positive correlation between serum levels of PFCs including PFOA, PFOS and PFHxS and self-reported food allergies has been reported [9]. However, there are very limited studies on the effects of PFCs on allergic reactions.

Mast cells are highly specialized cell type which plays a central role in allergic diseases. The activation of mast cells occurs via IgE/Ag-dependent and –independent manner. IgE-mediated mast cells activation involves the activation of multiple signaling pathways including phospholipase Cγ (PLCγ), mitogen-activated protein kinases, Akt and NF-κB and consequently, release of preformed mediators by degranulation and *de novo* synthesis of lipid mediators such as PGD_2 and LTC_4 and cytokines, leading to allergic inflammatory responses [10, 11].

In the present study, we have examined the effects of short-term exposure to PFCs on allergic responses using bone marrow-derived mast cells (BMMCs) and anaphylactic model. Their effects on mast cell activation were compared to that by bisphenol A (BPA), an endocrine disrupting chemical, which has been reported to activate mast cells [12,13].

Materials and Methods

Preparation of mouse BMMCs. BMMCs were isolated from 6~7-wk-old male Balb/cJ, as described previously [14]. Briefly, BMMCs were cultured in RPMI 1640 medium containing 10% (v/v) FBS, 100 U/ml penicillin, 10 mM HEPES, 100 μM MEM non-essential amino acid solution (Invitrogen) and 20% (w/v) PWM-SCM (pokeweed mitogen-spleen cell conditioned medium) as a source of IL-3. For cell stimulation, 1 x 10^6 cells/ml

were treated with 30 μM of PFOA, PFOS, PFHxS and 50 μM BPA for 1 h. DMSO was used as a vehicle control.

Immunoblotting. Cells were washed twice with ice-cold PBS and lysed in SDS-sample buffer Lysates were centrifuged at 14,000*g* for 20 min at 4°C and resulting supernatants were subjected to immunoblotting.

ß-hexosaminidase release assay. Mast cell degranulation was evaluated by measuring ß-hexosaminidase release as described previously [15].

PGD_2 and LTC_4 measurement. The levels of PGD_2 and LTC_4 following mast cell activation were quantified with respective immunoassay kits (Cayman Chemicals, Ann Arbor, Mich).

Local and systemic anaphylaxis in mice. Passive cutaneous anaphylaxis (PCA) was carried out by the protocol as described previously with minor modification [14]. Briefly, PFOS (4.56 mg/kg) and bisphenol A (3.25 mg/kg) were intradermally injected into one ear of 7-wk-old male mice. One hour later, mice were treated intravenously with 200 μl of PBS containing 1 % Evans blue (n=6 mice per group). After 1 h, blood was collected by cardiac puncture to determine serum LTC_4 and PGD_2 levels as described above and Evans blue from ear tissue was extracted with formamide at 63°C overnight and quantified by absorbance at 630 nm.

Results and Discussion

To determine the direct effects of PFCs on mast cell activation, mast cell degranulation and production of PGD_2 and LTC_4 were measured. BPA was treated as a positive control for mast cell activation. As reported by others [12,13], BPA significantly increased ß-hexosaminidase (ß-Hex), PGD_2 and LTC_4 levels. Similarly, PFOS significantly increased ß-Hex, PGD_2 and LTC_4 releases whereas the effects of PFOA and PFHxS were much less than that of PFOS (Fig 1). These results suggested that short-term PFOS exposure significantly increased mast activation in IgE/Ag-independent manner similar to BPA.

The activation of mast cells upon IgE/Ag stimulation involves multiple signaling pathways leading to degranulation and synthesis of eicosanoids. These signaling pathways stimulated by cross-linking of FcεRI upon IgE/Ag binding are well established and include phosphorylation of PLCγ, PLA2, Akt, ERK, JNK and p38 MAPK. It has been shown that the activation of mast cells by BPA was mediated by ERK and p38 MAPK [12]. Consistently, both PFOS and BPA increased the phosphorylation of ERK and p38 MAPK (Fig 2A). In addition, PFOS and BPA increased the activities of PLCγ, cPLA2, Akt , JNK and IKK ERK (Fig 2A). The increased ß-Hex, PGD_2 and LTC_4 levels by PFOS and BPA were markedly reduced by inhibitors of ERK pathway (U0126), Akt (Wortmanin) and p38 MAPK (SB203580) (Fig 2B-2E). This result suggests PFOS may share common signaling pathways involved in IgE/Ag stimulated mast cell activation. .

The mast cell-mediated allergic reactions are accompanied by vascular permeability, edema, etc. The allergenic effects of PFOS and BPA were further confirmed by local and systemic anaphylaxis using mouse

model. The cutaneous injection of PFOS and BPA showed greater vascular permeability than DMSO treated control, which was measured by dye extravasation (Fig 3A). Consistently, PFOS and BPA increased serum LTC_4 (Fig 3B) and PGD_2 (Fig 3C). Therefore, these results indicated that PFOS and BPA induced mast cell-activated allergic reaction in *in vitro* and *in vivo* models and the effect of PFOS was greater than either PFOA or PFHxS.

References

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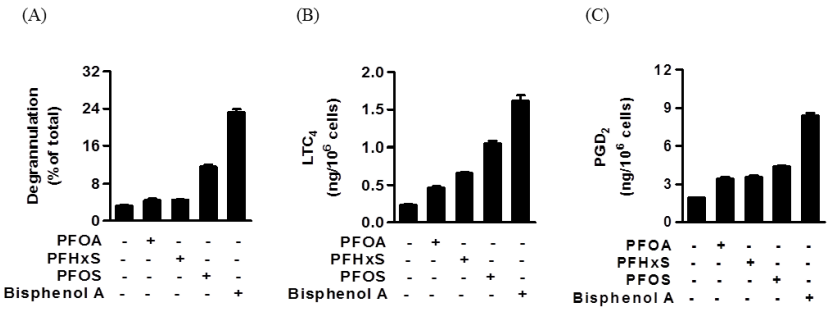

Fig1. The effects of PFCs and BPA on mast cell activation

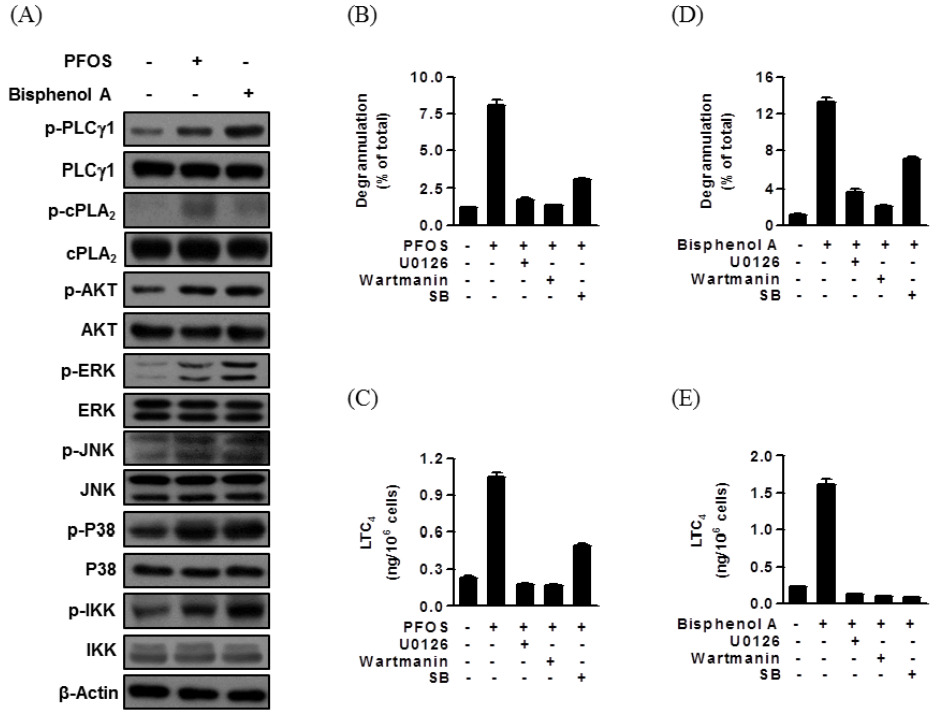

Fig2. The signaling pathways involved in PFOS- and Bisphenol A-induced mast cell activation

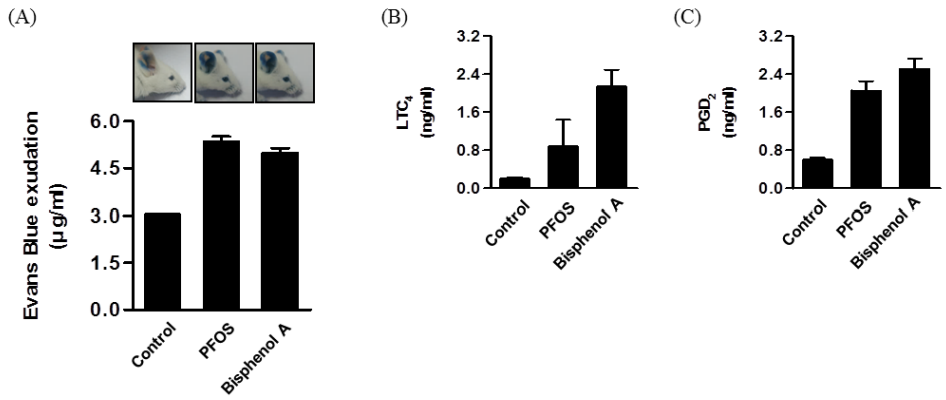

Fig3. PFOS and Bisphenol A-induced anaphylaxis