Pro- and anti-allergic effects of perfluorooctane sulfonate

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1 Introduction

Perfluoroalkyl compounds (PFCs) are widely distributed persistent organic pollutants (POPs). Due to their extreme stability, PFCs are accumulated in environment and in human via food web[1] and have been suggested to induce immune dysfunction. Several epidemiological studies reported diverse effects of PFCs on the development of allergic diseases. A Taiwanese case-control study has shown the positive association between serum levels of PFCs and the incidence of asthma in adolescents[2]. Consistently, serum PFCs including PFOS and PFHxS have been shown to be associated with asthma and nickel allergy[3] and with self-reported food allergies in adolescents[4]. In contrast, other case-control studies showed the inverse association of maternal PFCs with allergic diseases in early childhood[5,6]. On the other hand, there are several studies reporting no association between prenatal exposure to PFCs and allergy-related disease in early childhood[7,8]. Therefore, the impact of PFCs on the pathogenesis of allergic diseases remains to be elaborated.

Mast cell is a specialized cell type which plays a central role in both allergic and non-allergic immune responses[9]. The pathogenesis of mast cell-mediated allergic diseases is well established. Mast cells are activated by diverse stimuli IgE/antigen (Ag)-dependent and –independent manners. The binding of IgE/Ag to FcεRI, the high-affinity IgE receptor, on mast cells induces cross-linking of FcεRI, which triggers the release of preformed mediators such as histamine, tryptase and β-hexosaminidase by degranulation and *de novo* synthesis of lipid mediators such as prostaglandin D_2 (PGD_2) and leukotriene C_4 (LTC_4)[10]. Extensive studies have established the signaling pathways involved in the regulation of FcεRI-mediated mast cell activation. Aggregation of FcεRI upon binding of IgE/Ag induces the activation of receptor associated tyrosine kinases such as Fyn and Lyn and subsequently activates spleen tyrosine kinase (Syk). Then, Syk activates downstream signaling molecules, including linker for activation of T cells (LAT), PI3K/AKT, phospholipase Cγ (PLCγ), mitogen-activated protein kinases (MAPKs), NF-κB and intracellular Ca^{2+}, which mediate inflammatory allergic responses[10,11]. In addition to IgE/Ag, mast cells are activated by IgE-independent manners. Bisphenol A (BPA), a well-known xenoestrogen, has been shown to induce activation of mast cells which was mediated by ERK and extracellular Ca^{2+} [12]. This suggests that IgE-dependent and –independent stimulation of mast cells share downstream signaling pathways

In the present study, we have examined the effects of short-term exposure to PFOS on IgE-dependent and -independent activation of bone marrow-derived mast cells (BMMCs) and the underlying mechanisms. The effects of PFOS were compared to those of BPA.

2 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 either PFCs alone for 1 h or BMMCs were stimulated with IgE/Ag in the presence or absence of PFOS. DMSO was used as a vehicle control.

Immunoprecipitation and Immunoblotting. For immnoprecipitation, cell lysates were prepared in modified lysis buffer [0.1% NP-40, 50 mM HEPES (pH 7.0), 250 mM NaCl, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 0.5 mM dithiothreitol]. Total cell lysates (1 mg protein equivalent) were incubated with various antibodies for 2 h at 4°C and the immune complexes were precipitated with 20 μl of protein A-Sepharose. The precipitates were then washed three times with ice-cold lysis buffer. The precipitates or total cell lysates were subjected to SDS-PAGE and immunoblotted with corresponding antibodies.

siRNA transfection. BMMCs were cultured for 16 h in serum-free medium and transfected with a DharmaFECT transfection reagent (Dharmacon) containing siRNA (100 nM per well) in 12-well plates. After 24 h, BMMCs were stimulated with either PFOS or BPA as above.

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, MI).

3 Results and Discussion

To assess the effects of PFCs and BPA on mast cell activation, degranulation, intracellular Ca^{2+} and generation of lipid mediators (LTC_4 and PGD_2) were measured after exposure of BMMCs to PFHxS, PFOA, PFOS or BPA. PFOA, PFOS and BPA significantly increased degranulation, as assessed by β-hexosaminidase release, compared with DMSO vehicle control (Fig 1A). Consistently, the level of intracellular Ca^{2+}, an essential signaling molecule for degranulation of mast cells, was significantly increased by PFOA, PFOS and BPA (Fig 1B). The generations of LTC_4 and PGD_2 were significantly increased by all PFCs tested and BPA (Fig 1C and 1D). Among PFCs tested, PFOS showed the highest effects. Consistently, Both PFOS and BPA substantially increased the phosphorylation of PLCγ1, AKT and ERK (Fig 2A). In addition, PFOS and BPA increased the phosphorylation of proximal tyrosine kinases, Fyn and Lyn as well as Syk and its substrate, LAT (Fig 2A). Genetic knockdown of Fyn, Lyn, or Syk with specific siRNAs suppressed PFOS- or BPA-stimulated degranulation, release of eicosanoids (LTC_4 and PGD_2), and increase of the intracellular Ca^{2+} level (Fig 2B – 2M). Consistent with *in vitro* observations using BMMCs, PFOS and BPA significantly increased the amount of dye extravasation in the ears (Fig 3A) and serum elevation of LTC_4 (Fig 3B) and PGD_2 (Fig 3C), implying that both PFOS and BPA induce IgE-independent mast cell activation *in vivo*.

The effects of PFOS on FcεRI-mediated mast cell activation was examined. IgE-sensitized BMMCs were stimulated DNP-HSA (Ag) in the presence of absence of PFOS. IgE/Ag stimulation increased degranulation, intracellular Ca^{2+} level, and release of eicosanoids (LTC_4 and PGD_2) which were largely suppressed by PFOS (Fig 4A-4D). This result indicates the antagonizing effect of PFOS on IgE/Ag-stimulated mast cell activation.

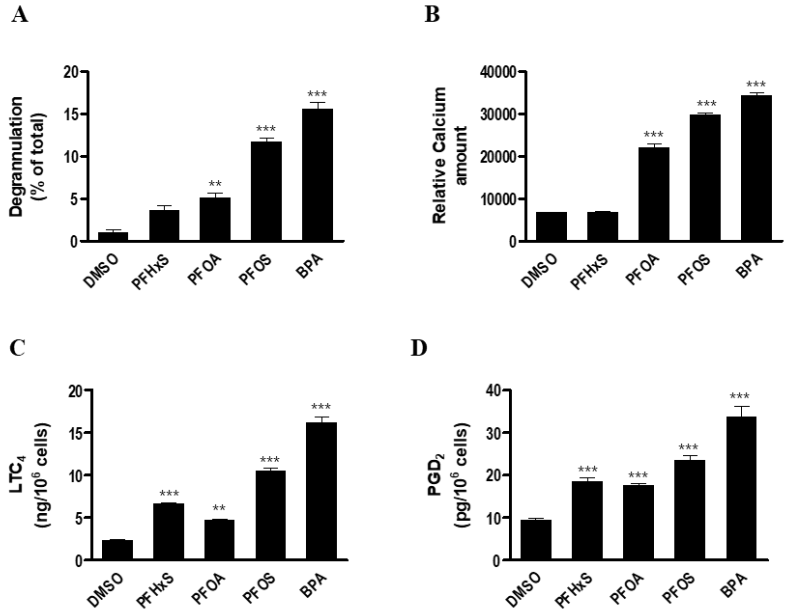

Figure 1: PFCs and BPA induced mast cell activation.

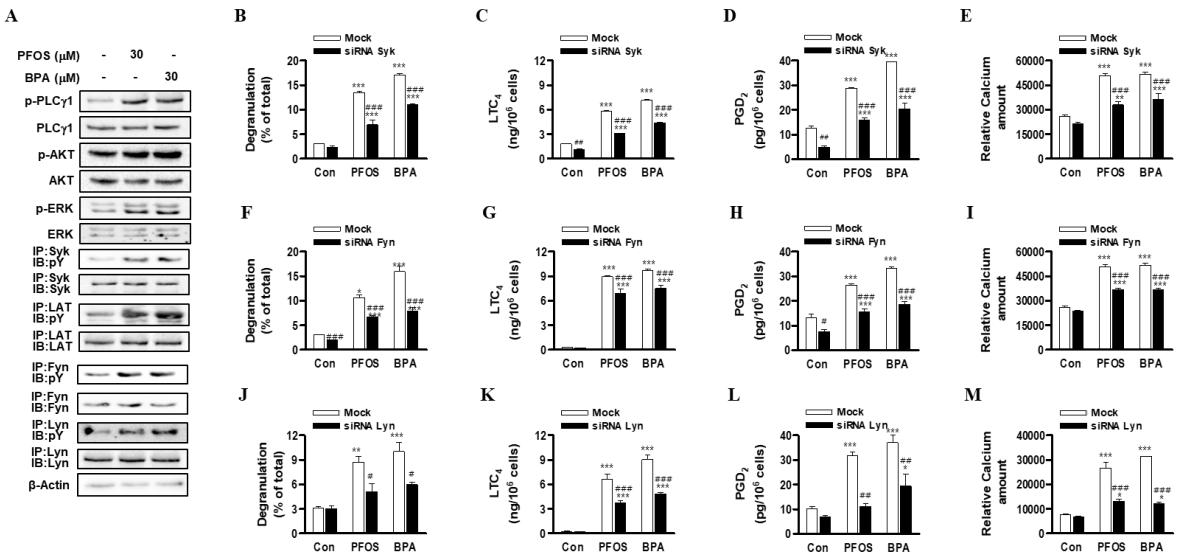

Figure 2: PFOS and BPA induce mast cell activation via Fyn, Lyn, and Syk pathway

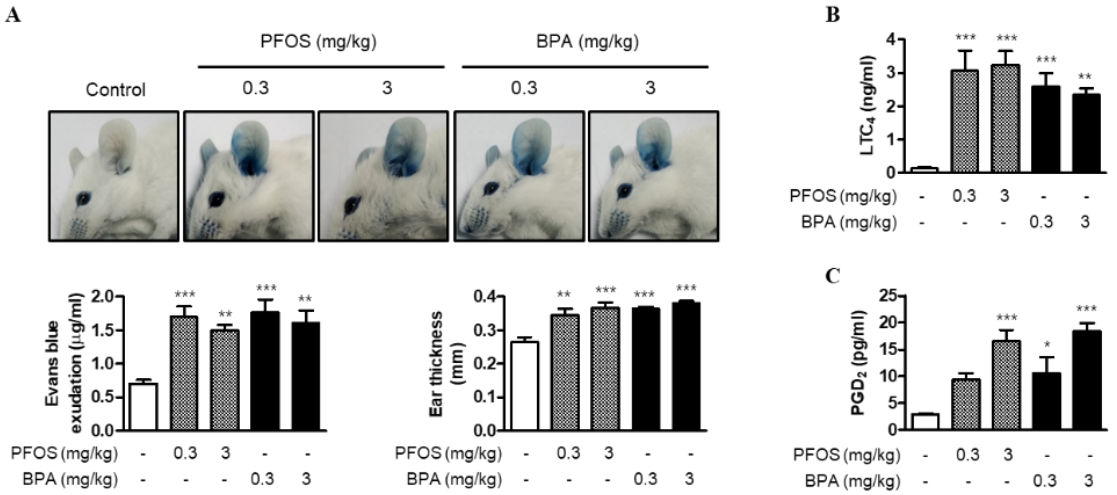

Figure 3: PFOS and BPA induce skin edema and inflammatory eicosanoid release

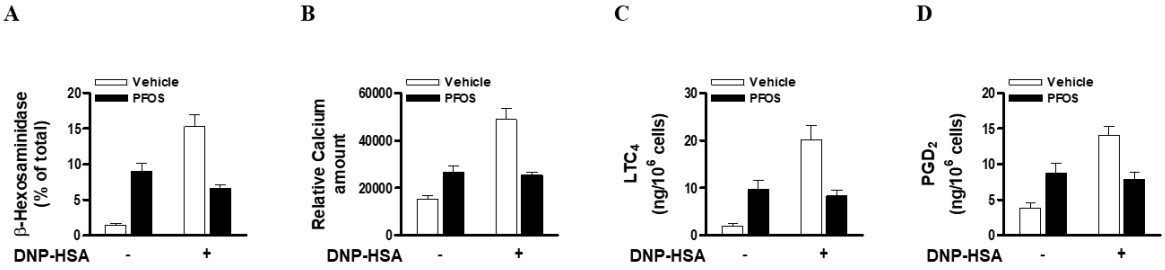

Figure 4: PFOS decreases IgE/Ag-stimulated mast cell activation

4 Conclusions

PFOS itself modestly augmented mast cell activation in *in vitro* and *in vivo* models whereas PFOS decreases FcεRI-mediated mast cell activation.

5 Acknowledgements

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