Perfluorooctanesulfonate (PFOS) activates mast cell-mediated allergic reaction

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Introduction

Millions of people worldwide are suffering from allergic diseases such as rhinitis, asthma, and atopic dermatitis. Many epidemiological studies have suggested that environmental contaminants are one of the important contributing factors for the pathogenesis of allergic disease [1,2]. In particular, exposure to environmental toxicants during perinatal period has been suggested to be responsible for the alteration in immune functions and increase development of allergic phenotype because this period is a critical time for the development of immune system and [3,4].

Perfluoroalkyl compounds (PFCs) have been widely used in industrial and various consumer products since 1950s. Due to their extreme stability, PFCs are accumulated in environment and accumulate in human via food web and are considered environmental pollutants [5]. Among these compounds, perfluorooctane sulfonate (PFOS) is the most extensively distributed and studied member of PFC family. PFOS has been detected in serum from general population as well as in umbilical cord and breast milk [6-8], indicating human exposure to PFCs during perinatal period. Recent animal and human studies have shown that PFCs have immunotoxic effects [9,10]. It has been reported that PFCs exposure reduced immune responses to childhood vaccination with rubella and booster vaccination with diphtheria and tetanus in adults [11,12]. In addition, positive correlation between serum levels of PFCs including PFOA, PFOS and PFHxS and self-reported food allergies has been reported [13]. However, there are very limited studies on the effects of PFCs on allergic reactions.

Mast cells play a central role in allergic diseases. The activation of mast cells occurs via IgE/Ag-dependent and –independent manner. The activation of mast cells involves the activation of multiple signaling pathways including phospholipase Cγ (PLCγ), mitogen-activated protein kinases, Akt and NF-κB, leading to release of preformed mediators by degranulation and *de novo* synthesis of lipid mediators and cytokines [14,15].

In the present study, we have examined the effects of short-term PFOS exposure on allergic using bone marrow-derived mast cells (BMMCs).

Materials and Methods

Preparation of mouse BMMCs. BMMCs were isolated from 6~7-wk-old male Balb/cJ, as described previously [16]. Briefly, BMMCs were cultured in RPMI 1640 medium containing 10% (v/v) FBS, 100 U/ml penicillin (Thermo Fisher Scientific), 10 mM HEPES (Sigma-Aldrich), 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 with IgE/Ag, 1 x 10^6 cells/ml were sensitized with 500 ng/ml mouse anti-DNP IgE (Sigma-Aldrich) overnight and then stimulated with 100 ng/ml DNP-HAS (Sigma-Aldrich) for 15 min at 37°C.

Immunoblotting. Cells were washed twice with ice-cold PBS and lysed in SDS-sample buffer containing 1% (v/v) NP-40, 50 mM Tris (pH 8.0), 150 mM NaCl, 1 mM EDTA, 1 mM sodium orthovanadate, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 2 μg/ml aprotinin, 2 μg/ml leupeptin, and 1 μg/ml pepstatin A for 30 min on ice. 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 [17].

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).

Results and Discussion

The activation of mast cells results in the release of various mediators responsible for the development of allergic inflammatory responses. The effect of PFOS on ß-hexosaminidase (ß-Hex) release was examined. BMMCs were treated with 30 μM of PFOS and 50 μM bisphenol A (BPA) for 1 h or stimulated with IgE/Ag for 15 min. IgE/Ag-stimulated mast cells (DNP-HAS) induced a robust increase in ß-Hex release (17 %). PFOS significantly increased ß-Hex release (7.8 %) compared to control (2.4 %). The effect of PFOS was compared to that of BPA, another environmental pollutant known as having immunotoxic properties. As reported by others [18,19], BPA also significantly increased ß-Hex release (5.1 %) (Fig 1A). The effect of PFOS was concentration dependent (Fig 1B). Similarly, PFOS significantly increased the synthesis of leukotriene C_4 (LTC_4) and prostaglandin D_2 (PGD_2) (Fig 2, 3). These results suggested that short-term PFOS exposure significantly increased mast cell degranulation via IgE/Ag-independent manner with comparable extent to that of BPA.

The signaling cascade involved in IgE/Ag-stimulated mast cell activation has been well established. The binding of IgE/Ag on mast cells induce the activation of proximal receptor-associated tyrosine kinases including Lyn, Fyn and Syk, followed by phosphorylation of PLCγ and PI3K. The activation of PLCγ promotes Ca^{2+} mobilization and protein kinase C (PKC) activation. PI3K converts PIP2 to PIP3, resulting in activation of various signaling molecules such as BtK, Akt and PDK1. These signaling pathways converge on the activation of downstream signaling pathways including MAP kinases (MAPK) and IKK- nuclear factor κB (NF-κB), which mediate degranulation and production of lipid mediators. Both PFOS (3 nM -30 μM) and BPA increased the activities of PLCγ, IKK, Akt , p38 and ERK (Fig 4). This result suggests PFOS may share common signaling pathways involved in IgE/Ag stimulated mast cell activation. .

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(A) (B)

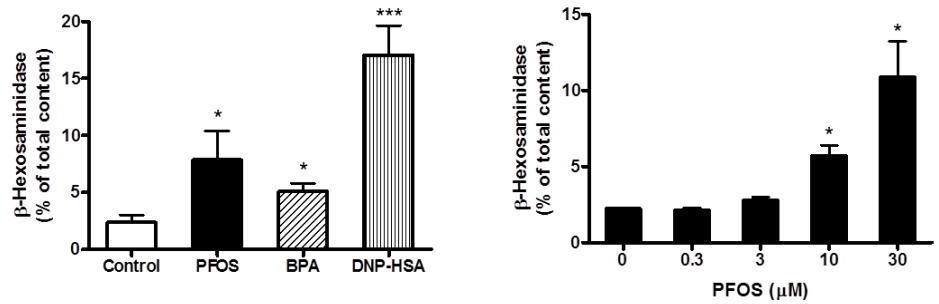

Fig1. The effects of PFOS, BPA and IgE/Ag (DNP-HAS) on mast cell degranulation

(A) (B)

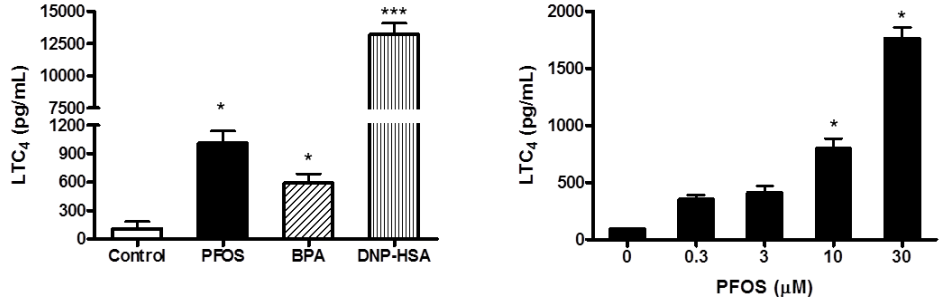

Fig2. The effects of PFOS, BPA and IgE/Ag (DNP-HAS) on LTC_4 release

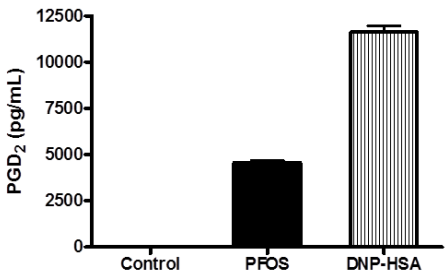

Fig3. The effects of PFOS and IgE/Ag (DNP-HAS) on PGD_2 release

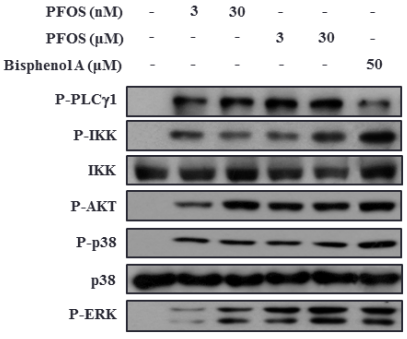

Fig4. The effects of PFOS and BPA on phosphorylation of signal molecules