Fyn- AND Syk-DEPENDENT ACTIVATION OF MAST CELLS BY PERFLUOROOCTANE SULFONATE

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Introduction

Perfluorooctane sulfonate (PFOS) is the most extensively distributed member of perfluorinated chemicals (PFCs), which have been widely used in industrial and various consumer products. Due to their extreme stability, PFCs are accumulated in environment and in human via food web[1]. Therefore, PFCs are considered ubiquitous environmental pollutants. Accumulative studies have suggested the diverse impacts of PFCs on human health including immunotoxic effects[2-4]. Previous study has reported the positive correlation between serum levels of PFCs including PFOS and childhood asthma in measles, mumps, and rubella (MMR)-unvaccinated group[5]. Consistently, serum PFCs have been shown to be associated with asthma and nickel allergy[6] and with self-reported food allergies in adolescents[7]. In contrast, there are several studies reporting no association between prenatal exposure to PFCs and allergy-related disease in early childhood[8,9]. Therefore, the impact of PFCs on immune function-related health outcomes is not yet conclusive.

Mast cells play a central role in allergic diseases. The activation of mast cells occurs via IgE/Ag-dependent and –independent manners. IgE-mediated mast cells activation is extensively studied and well established. The binding of IgE/Ag to FcεRI, the high-affinity IgE receptor, activates receptor associated tyrosine kinases including Fyn and Lyn and subsequently activates spleen tyrosine kinase (Syk), a central kinase of mast cell activation. Then, Syk activates multiple downstream signaling pathways including phospholipase Cγ (PLCγ), mitogen-activated protein kinases, Akt and NF-κB, which consequently induce the 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]. However, the signaling pathways involved in IgE-independent activation of mast cells are not much studied.

In the present study, we have examined the effects of PFOS on mast cell activation and the involved signaling pathways using bone marrow-derived mast cells (BMMCs) to elucidate the possible role of PFOS in the development of allergic diseases. The effect of PFOS 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.

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).

Results and Discussion:

It has been reported that BPA-induced degranulation of mast cells was ERK and Ca^{2+} dependent (O'Brien et al., 2014). Since these signaling pathways are known to play an important role in FcεRI-dependent activation of mast cells, we first examined the involvement of signaling pathways required for FcεRI-mediated mast cell activation to elucidate the underlying mechanism of PFOS-induced BMMC activation. Both PFOS and BPA substantially increased the phosphorylation of PLCγ, AKT as well as ERK (Fig 1A). This effect of PFOS was concentration-dependent. Consistent with the positive effects of PFOS and BPA on FcεRI signaling molecules, PFOS- and BPA-induced degranulation (Fig 1B and 1E), production of LTC_4 (Fig 1C and 1F) and PGD_2 (Fig 1D and 1G) were significantly reduced by U0126 and wortmannin, inhibitors of ERK pathway and PI3K/AKT pathway, respectively, suggesting that PFOS- and BPA-increased mast cell activation is mediated by ERK and AKT. Next, we have examined the effects of PFOS and BPA on the activation of Syk, an upstream kinase of PLCγ, AKT and ERK in FcεRI-mediated mast cell activation. As expected, tyrosine phosphorylation of Syk was increased by PFOS and BPA. Interestingly, PFOS and BPA increased the phosphorylation of Fyn, a FcεRI-associated tyrosine kinase (Fig 1H). Thus, it appears that the mechanistic action of IgE-independent activation of mast cells by PFOS and BPA share the signaling components with FcεRI–mediated signaling.

Since both PFOS and BPA increased the phosphorylation of Fyn and Syk in BMMCs, we examined the roles of these tyrosine kinases in mast cells using Fyn- and Syk-specific siRNAs. As expected, Fyn siRNA inhibited the phosphorylation of Fyn and its protein level compared to control siRNA (Mock) (Fig 2A). Additionally, Fyn siRNA effectively reduced the phosphorylation of PLCγ, AKT and ERK induced by PFOS and BPA. In consistent with the inhibitory effects of Fyn siRNA on signaling molecules, PFOS- and BPA-increased degranulation (Fig 2B), generation of LTC_4 (Fig 2C) and PGD_2 (Fig 2D), and intracellular Ca^{2+} (Fig 2E) were significantly reduced by Fyn knockdown. Similarly, Syk siRNA effectively reduced the phosphorylation of Syk, PLCγ, AKT and ERK as well as Syk protein level (Fig 3A). Consistently, PFOS- and BPA-increased degranulation (Fig 3B), generation of LTC_4 (Fig 3C) and PGD_2 (Fig 3D), and intracellular Ca^{2+} (Fig 3E) were significantly reduced by silencing of Syk. These suggest that the activation of BMMCs by PFOS and BPA involves Fyn and Syk activation, which act as upstream kinases of PLCγ, AKT and ERK.

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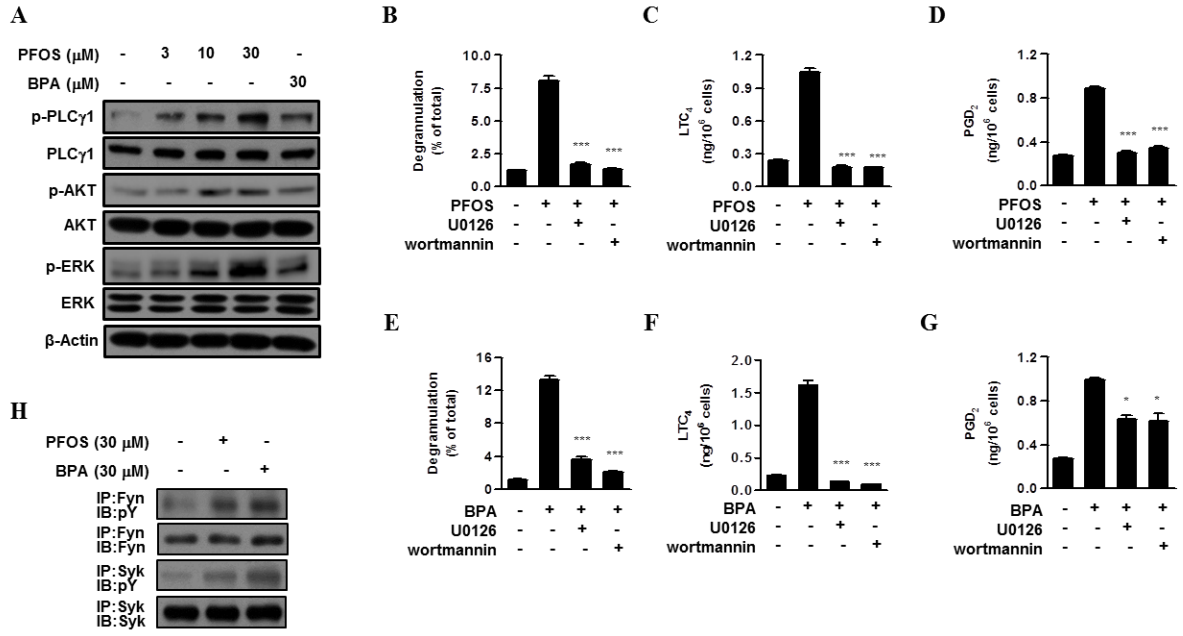

Fig1. The signaling pathways involved in PFOS- and Bisphenol A-induced mast cell activation

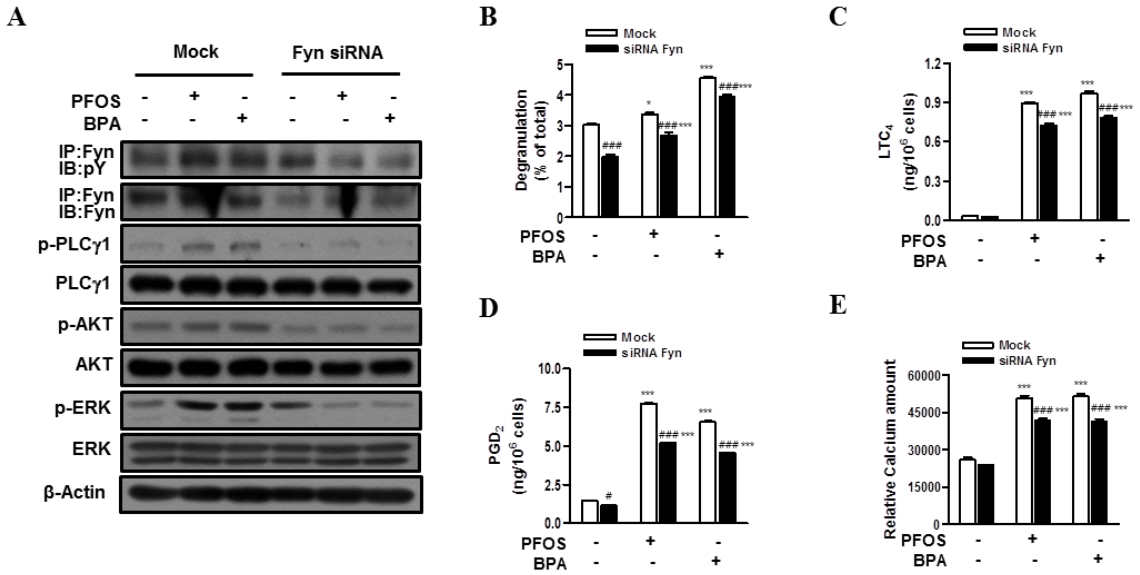

Fig2. The role of Fyn in PFOS- and Bisphenol A-induced mast cell activation

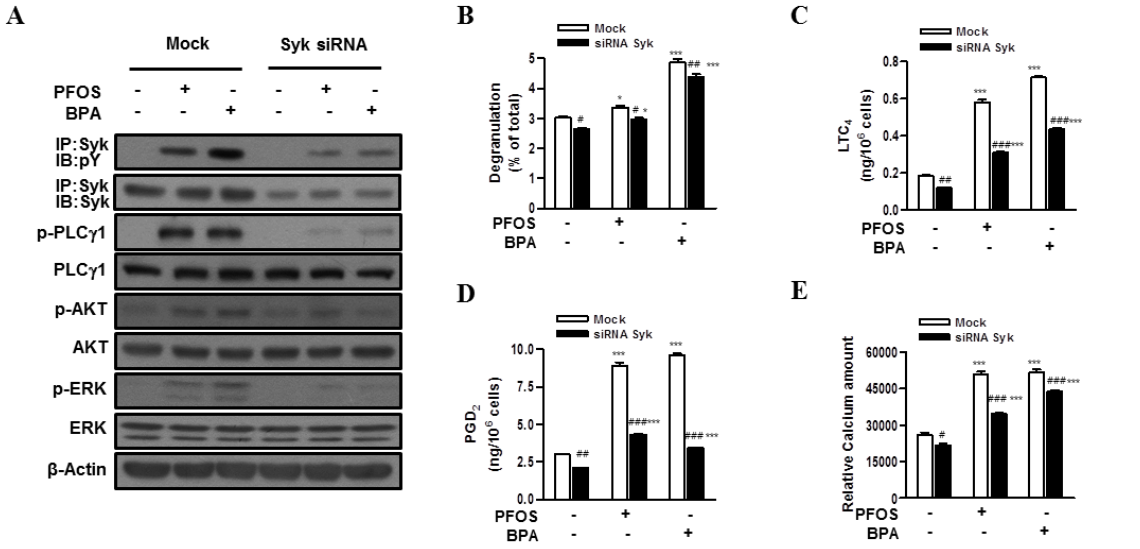

Fig3. The role of Syk in PFOS- and Bisphenol A-induced mast cell activation