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PERFLUORINATED COMPOUNDS: EMERGING POPs WITH POTENTIAL IMMUNOTOXICITY

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Abstract

Perfluorinated compounds (PFCs) have been recognized as an important class of environmental contaminants commonly detected in blood samples of both wildlife and humans. These compounds have been in use for more than 60 years as surface treatment chemicals, polymerization aids, and surfactants. They possess a strong carbon-fluorine bond, which leads to their environmental persistence. There is evidence from both epidemiology and laboratory studies that PFCs may be immunotoxic, affecting both cell-mediated and humoral immunity. Reported effects of PFCs include decreased spleen and thymus weights and cellularity, reduced antibody production, reduced survival after influenza infection, and altered cytokine production. Immunosuppression is a critical effect associated with exposure to PFCs, as it has been reported to reduce antibody responses to vaccination in children. Mounting evidence suggests that immunotoxicity in experimental animals can occur at serum concentrations below, within, or just above the reported range for highly exposed humans and wildlife. Considering bioaccumulation and exposure to multiple PFCs, the risk of immunotoxicity for humans and wildlife cannot be discounted. This review will discuss current and recently published work exploring the immunomodulatory effects of PFCs in experimental animals and humans.

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Conflict of Interest

For the past 3 years since the beginning of this work, all Authors declare of not having any financial, personal, or association with any individuals or organizations that could have inappropriately influenced the submitted work.

Keywords

perfluorinated compounds; immunosuppression; PPAR- α receptor; cytokine; vaccination

1. Uses, environmental and serum concentrations of PFCs

Perfluorinated compounds (PFCs), such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), have been produced since the 1950s and are used in many industrial and manufacturing applications, including production of nonstick cookware, waterproof and breathable textiles, and protective coatings for paper, food packing materials, and carpets. PFCs comprise a heterogeneous class of chemicals consisting of an alkyl chain (4–14 carbons), which is partially or fully fluorinated, and have different functional groups attached. Among the PFCs are the perfluoroalkyl carboxylic acids (i.e. PFOA, PFDA), perfluoroalkyl sulfonic acids (i.e. PFOS, PFBS), perfluoroalkyl sulfonamides (i.e. PFOSA), and other polyfluorinated compounds, such as fluorotelomer alcohols (i.e. fluorotelomer). PFCs are commonly detected in wildlife and human blood samples (Suja et al., 2009; Rayne and Forest, 2009). Food intake appears to be the major factor contributing to background PFC levels in human sera, while exposure to contaminated water and soils results in elevated levels in both human and wildlife populations (Vestergren and Cousins, 2009).

These compounds are present in surface, ground, marine, and drinking waters at concentrations that vary from pg/l to μ g/l and in soils at levels up to approximately 50 ng/g of dry matter. Some wastewaters contain PFCs at mg/l to low g/l levels (Rayne and Forest, 2009; Zareitalabad et al., 2013). Although western manufacturers began a voluntary phase out of PFOS and PFOA production in 2002, both PFOS and PFOA persist in the environment because of extremely stable carbon-fluorine bonds and circulate through environmental media including air, soil, plants and food stocks (Davis et al., 2007, Domingo, 2012).

The PFOA elimination half-life in serum markedly differs among species and between sexes, being 1 to 9 days in rats, 18 days in mice, 20 to 40 days in dogs, 20 to 32 days in monkeys, and 3.8 years in humans (Calafat et al., 2007). Blood samples of occupationally exposed individuals and the general human population in various countries were found to contain PFOS and PFOA at measurable levels. In the United States, the mean serum concentration in the general population was reported as 20.7 ng/ml for PFOS and as 3.7 ng/ml for PFOA (Calafat et al., 2007), with children often showing higher serum concentrations than adults (Kato et al., 2009). One explanation for the apparent greater mean concentrations of PFCs in children than in adults is increased exposure among children resulting from increased contact with carpeted floors and upholstered furniture coupled with hand-to-mouth activity (Kato et al., 2009). PFOA serum concentrations reported in occupationally exposed humans were between 428 and 12000 ng/ml (Steenland et al., 2010), and between 145 and 3490 ng/ml for PFOS (Olsen et al., 2007).

2. Toxicology findings

In the context of toxicology, PFOA and PFOS have attracted most attention because they represent a high proportion of environmental PFCs. Adverse effects reported in experimental animals exposed to PFOA or PFOS include hepatomegaly and hepatic peroxisome proliferation; liver, testicular, and pancreatic tumors; reproductive and developmental deficits, including reduced fetal weights, skeletal and cardiac malformations; neurotoxicity; and immunotoxicity. Data from experimental animals demonstrate that the perfluorinated alkyl acids induce peroxisomal proliferation, hepatomegaly, altered steroidogenesis, and body weight loss associated with a wasting syndrome (Kennedy, 2004; Pastoor et al., 1987; Liu et al., 1996b; Olsen et al., 1999; Biegel et al., 2001; Lau et al., 2007). Ammonium perfluorooctanoate, the ammonium salt of PFOA, produces increased aromatase activity and plasma estradiol levels, and induces Leydig cell adenomas (Liu et al., 1996a, 1996b).

The peroxisome proliferator-activated receptors (PPAR) belong to the nuclear hormone receptor superfamily, and there are three primary subtypes: PPAR α , β/δ , and γ . These receptors regulate important physiological processes that impact lipid homeostasis, inflammation, adipogenesis, reproduction, wound healing, and carcinogenesis (Chinetti et al., 2000). PFC-associated toxicity in laboratory rodents has been related to ligand activation of PPAR α (Peraza et al., 2006). It is, however, important to stress that there are important species differences in receptor-specificity, receptor activity and related ligand binding/activation, which are all critical factors that mechanistically influence susceptibility to PFC toxicity. For example, PPAR α -humanized mice do not exhibit the liver toxicities found in wild-type mice (Cheung et al., 2004). The hepatocarcinogenic effect of PPAR α ligands in rodents is one of the best examples of such a species-specific effect: epidemiological studies of humans taking fibrates (a potent class of PPAR α agonists) to control serum lipid levels found no evidence of increased cancer, even though all rodents exposed to the same compounds develop liver tumors (Gonzales et al., 1998). Therefore, the role of species differences in signal transduction in extrapolating animal data to human risk assessment needs to be carefully considered.

In spite of a number of experimental and epidemiological studies, the health effects of PFCs in humans still remain controversial (Butenhoff et al., 2004; Emmett et al., 2006; Steenland et al., 2010). Epidemiological studies on large cohorts of occupationally exposed workers, mainly from manufacturing sites in the US (3M and DuPont) in the period 1947–2002, found no association between PFOA exposure and significant adverse health effects (Ubel, 1989; Gilliland Mandel, 1993; Alexander, 2001; Alexander and Olsen, 2007; Leonard et al., 2008). The slight excess of prostate cancer reported by Gilliland and Mandel (1993) and of bladder cancer found in the study by Alexander (2001) was not confirmed in two occupational cohorts or a general population study (Leonard et al., 2008; Eriksen et al., 2009; Lundin et al., 2009). In a comprehensive review, Steenland et al. (2010) evaluated the epidemiologic literature on PFOA and its association with several health outcomes, including immunomodulation. The authors concluded that the most consistent health findings were modest increases in cholesterol, which may increase the risk of cardiovascular diseases, and elevated serum uric acid. The later finding was associated with an increased

risk of chronic kidney disease in the study cohort, although the relationship between elevated uric acid and kidney disease is not absolute (Cain et al., 2010). The mechanism by which PFCs are related to changes in serum cholesterol in humans is unknown, but the finding of positive associations between perfluorinated compounds and altered cholesterol levels contradicts what would be expected from animal studies. In animals, PFOA is a strong PPAR α activator in the liver and this proliferation has been shown to alter lipid metabolism with decreases, not increases, in serum lipids (Kennedy et al., 2004). Peroxisome proliferation is much less apparent in humans (Palmer et al., 1998) and may clarify the discrepancy between human and animal studies on the associations between PFCs and cholesterol levels.

In humans, the possible interference of PFCs with metabolism of fatty acids should not be underestimated and deserves further investigations on the mechanisms of action and as a possible risk factor for metabolic disorders and/or cardiovascular diseases (Costa et al., 2009). Certain chronic diseases with an inflammatory component, including diabetes, heart disease, and stroke, have been reported to be elevated in studies of exposed populations; however, the evidence is too meager and inconsistent for solid conclusions (Steenland et al., 2010).

3. Evidences of immunotoxicity in rodents

In a review of the U.S. Environmental Protection Agency (US EPA) risk assessment of PFOA and its salts by the US EPA Science Advisory Board, immunotoxicity was mentioned as an endpoint of concern for PFCs ([http://yosemite.epa.gov/sab/sabproduct.nsf/ded77e69fc8ced288525711e006fe16b/\\$file/kropp-ewg.pdf](http://yosemite.epa.gov/sab/sabproduct.nsf/ded77e69fc8ced288525711e006fe16b/$file/kropp-ewg.pdf)). Early studies with mice indicated that ingestion of feed containing 0.02% PFOA by weight (approximately 30 mg/kg bw/day) suppressed antigen-specific IgM antibody production, caused thymic and splenic atrophy, and altered T-cell populations (Yang et al., 2000, 2001, 2002a, 2002b). It is important to note that at this dose, overt toxicity, as indicated by body weight loss, was observed, and, therefore, the observed immunosuppressive effects could be secondary to severe toxicity rather than a specific effect of PFOA on the immune system (Loveless et al., 2008; Fang et al., 2008). However, DeWitt et al. (2008) demonstrated that suppression of the antigen-specific IgM antibody response occurs at doses that produce no signs of acute toxicity or effects on spleen weight. Furthermore, this group (DeWitt et al. (2009a) determined that adrenalectomy did not protect mice from the immunosuppressive effects of PFOA at doses that reduced body weight gain. Although the HPA axis may contribute to immunotoxicity at high doses in intact animals, suppression of humoral immunity at lower doses (e.g., 3.75 and 7.5 mg PFOA/kg PFOA) is independent of elevated corticosterone and therefore represents a true immunotoxic effect (DeWitt et al., 2009a). DeWitt et al. (2008) calculated a benchmark dose for immunosuppression, based on 3.75 mg PFOA/kg as a lowest observed adverse effect level (LOAEL), an exposure that resulted in 7.4×10^4 ng PFOA/ml of serum measured one day post-exposure. This concentration is approximately 150 times lower than that reported in highly exposed individuals living near a PFOA production facility, 6 times greater than concentrations observed in highly exposed humans (12 μ g/ml) and 20,000 times greater of the mean serum concentration measured in the general population (3.7 ng/ml).

Recent studies have shown that PFOS affects antibody production in mice at levels found in the general human population (Peden-Adams et al., 2008). Peden-Adams et al. (2008) reported that serum concentrations of PFOS associated with the LOEL (lowest observed effect level) for suppression of humoral immunity were 91.5 ng/ml for males and 666 ng/ml for females. Additionally, PFOS exposure (5 or 25 µg/kg PFOS/kg/d) suppressed immunity to influenza A infection in mice without altering body or lymphoid organ weights, resulting in significant increases in emaciation and mortality (Guruge et al., 2009). The pesticide sulfluramid, which is rapidly metabolized to PFOS, also has been demonstrated to target T-dependent, IgM antibody production in mice at exposure levels 10-fold lower than overtly toxic doses (Peden-Adams et al., 2007), further confirming the immune system as a sensitive target of PFC toxicity. Interestingly, the immune system of nonmammalian species has also been shown to be affected at current environmental levels of PFCs (Peden-Adams et al., 2009)). In contrast, Qazi et al. (2010) reported that 28 days of dietary exposure to PFOS did not affect IgM TDAR in B6C3F1 male mice at serum concentrations 8- to 85-fold greater than occupationally exposed humans. Similarly, Lefebvre et al. (2008) indicated that when Sprague-Dawley rats were given PFOS in their diet for 28 days, IgG TDAR was not statistically suppressed, although they did not measure IgM TDAR. Regardless of the presence of some conflicting data, the weight of evidence clearly supports the conclusion that antibody production is an endpoint sensitive to modulation by PFCs.

4. Evidences of immunotoxicity in humans

Data on the immunotoxicity of PFCs in humans are limited; however, they seem to suggest that exposure to PFCs may be associated with immunosuppressive effects. Emmett et al. (2006) reported a slight increase in absolute monocyte counts of residents who lived in a water district contaminated with PFOA, while there was no significant relationship between serum PFOA and the percentage of monocytes in differential white cell counts. The C8 Health Project, which collected data from residents living in the vicinity of a PFOA plant, found that immunoglobulins (Ig) A and E (female only) and C reactive protein (CRP) levels decreased with increasing blood levels of PFOA and antinuclear antibodies increased with increasing PFOA serum concentration (Fletcher et al., 2009). Brieger et al. (2011) explored the impact of PFOS and PFOA on selected functions of human leukocytes in vitro, and reported that PFOA and PFOS were associated with reduced NK cell activity and reduced the release of the pro-inflammatory cytokine TNF- α following lipopolysaccharide (LPS)-stimulation.

Age, gender, genetic background, and social factors typically influence the response to immunotoxic compounds, resulting in a great deal of variability in biological outcomes. Thus, exposure-related alterations in immune functions that are well tolerated by “normal” individuals could have more serious consequences in those individuals who are chronically ill, or malnourished, in the very young, whose immune systems have yet to complete development, or in the elderly, whose immune systems are in decline (Germolec, 2004; Holsapple et al., 2004; Dieter, 2008). Two recently published studies indicate that PFCs may lower vaccine protection in children (Grandjean et al., 2012; Granum et al., 2013). The antibody response to immunization is considered a feasible parameter to evaluate in population studies; it is clinically relevant and reflects immune system functionality (van

Loveren et al., 1999; Luster et al., 2005; WHO, 2012). In laboratory rodents, the antibody response to T cell-dependent antigens is considered one of the most sensitive and predictive measures of immune function currently in use, as it requires T cells, B cells, and antigen-presenting cells to function properly to elicit an antibody response (Luster et al. 1992) and correlates well with resistance to infection (Luster et al., 1993). Therefore, reduced vaccine responses in children may reflect impacts on a sensitive immunotoxicological endpoint in a sensitive subpopulation.

In a prospective study of a birth cohort from the National Hospital in the Faroe Islands involving a total of 587 children, Grandjean et al. (2012) found that five to seven year old children with high serum concentrations of PFCs did not respond as well to diphtheria and tetanus immunizations as children with lower PFC levels: a 2 fold increase in serum PFC concentrations at 5 years of age was reported to reduce responses to tetanus and diphtheria booster immunizations by half. The fishing community of the Faroe Islands was chosen due to the frequent intake of marine food, which is associated with increased exposure to PFCs (Weihe et al., 2008). The mean maternal serum concentrations of PFOS and PFOA were 27.3 ng/ml and 3.2 ng/ml, respectively, while in five year old children serum concentrations were 16.7 ng PFOS/ml and 4.06 ng PFOA/ml. Childhood immunizations began with vaccination at age 3 months against diphtheria and tetanus, along with pertussis, polio, and *Haemophilus influenzae* type B. Repeat inoculations were given at 5 and 12 months of age, with a booster vaccination against diphtheria and tetanus at age 5 years. To evaluate the long-term antibody response to the vaccinations, the birth cohort underwent prospective follow-up until age 7 years. Examinations took place at age 5 years prebooster (587 children), approximately 4 weeks after the booster, and at age 7 years (464 children). Prenatal exposure to five selected PFCs was assessed by analyses of serum obtained from the mother at the last antenatal examination at week 32 of gestation; postnatal exposure was assessed by analysis of serum from the child at age 5 (prebooster). Multiple regression analyses with covariate adjustment showed that prenatal exposures to both PFOS and PFOA were negatively associated with antidiphtheria and antitetanus antibody concentrations. The authors concluded that elevated PFC concentrations, particularly of PFOS and PFOA, were responsible for antibody responses to vaccination below those associated with effective protection for tetanus and diphtheria (0.1 IU/ml). This study was the first to link PFC exposures in children to deficits in immune system functions.

In a second prospective study, involving the birth-cohort BraMat, Granum et al. (2013) found an inverse association between the level of anti-rubella antibodies in children at age 3 years and maternal plasma concentrations of PFCs. The antibody levels specific for the four vaccines included in the Norwegian Childhood Vaccination Program, included measles, rubella, tetanus and *Haemophilus influenzae* type B were assessed. Clinical health outcomes were collected using a specific questionnaire administered to the participants. The mean maternal serum concentrations were 1.1 ng/ml for PFOA and 5.5 ng/ml for PFOS. In multivariate models, increased concentrations of PFCs in maternal blood were significantly associated with reduced anti-rubella antibodies in children at age 3 years, while no significant associations were found between the concentrations of PFCs and antibody titers to the other vaccines. In addition, these investigators found a positive association between

the maternal concentration of PFOA and PFNA (perfluorononanoate) and the number of episodes of common cold for the children in this cohort, and between maternal PFOA and PFHxS (perfluorohexane sulfonate) concentration and the number of episodes of gastroenteritis. This study indicates that pre-natal exposure to PFCs is associated with immunosuppression in early childhood with effects on both response to pediatric vaccine and immune-related clinical outcomes.

In a study involving 1400 pregnant women and their offspring, characterized by mean maternal serum concentrations of 5.6 ng/ml for PFOA and 35.3 ng/ml for PFOS during the first trimester, Fei et al., (2010) did not find an association between PFC exposure and increased risk of hospitalization for infectious diseases in children followed for up to 11 years, using data from the Danish National Birth Cohort. The outcome of interest was any hospitalization due to infections in early childhood. The number of hospitalizations was counted for each child during the follow-up period, which began on the date of birth and ended on the date of death, emigration, or December 31, 2008, whichever occurred first. Out of 1400 children, 363 (25.9%) were hospitalized at least once during the follow-up period due to infectious diseases. If considered all together, children who were prenatally exposed to higher levels of PFOA had lower risks of hospitalization due to infections, but the incidence rate ratio (IRR) was statistically significant only for the second quartile of exposure [IRR1/40.71, 95% confidence interval (CI) 0.53–0.94] compared with the first. No significant association was observed for PFOS. However, if children were stratified by sex, girls had a higher risk of hospitalization for infections associated with higher maternal PFOS or PFOA levels, with the IRRs ranging from 1.14 to 1.61 for PFOS and 1.20 to 1.74 for PFOA in the higher three quartiles compared with the lowest. In contrast, the IRRs were all below 1.0 among boys. This sex difference is of interest, and it is consistent with *in vitro* data we recently published (Corsini et al., 2012): in terms of cytokine production, leukocytes obtained from female donors appear to be more sensitive to the immunosuppressive effects of PFCs compared to leukocytes from male donors. Interestingly, sex also significantly modified the positive association between PFOA and cholesterol, with a stronger association among women as demonstrated in a middle-aged Danish population (Eriksen et al., 2013).

While the majority of published human data are indicative of immunosuppressive effects of PFCs, especially in children, the role of PFCs in allergic diseases is still not well-known. T-helper (Th)-1 and Th2 cells play specific roles in immunity. Th1 cells promote cell-mediated immunity against intracellular pathogens, and the prototypical cytokines secreted are IFN γ and IL-2. Th2 cells are involved in humoral immunity and secrete IL-4 to promote production of immunoglobulins. If cytokine profiles are shifted toward one subset over another, the risk of immune dysfunction increases. For example, in separate studies, Dong et al. (2011) and Zheng et al. (2011) evaluated the effect of short-term or subchronic PFOS exposure on the balance between Th1 and Th2 cytokines in C57BL/6 male mice. Results indicated that the cytokine balance favored Th2 responses in experimental animals (Zheng et al. 2011; Dong et al. 2011).

In a study by Wang et al. (2011) involving 244 children and investigating correlations among cord blood IgE and serum total IgE at 2 years of age and cord blood PFC levels, prenatal PFOA and PFOS exposures positively correlated with cord blood IgE levels in

boys. In contrast, in a study by Okada et al. (2012), cord blood IgE levels decreased significantly with high maternal PFOA levels among female infants, and no relationship was found between maternal PFOS and PFOA levels and infant allergies and infectious diseases as assessed from self-administered questionnaires at 18 months of age. In agreement, the study by Granum et al. (2013) showed no significant associations between prenatal exposure to PFCs and allergy. Further studies are necessary to definitively exclude or include a role of PFCs in allergy.

5. Mechanisms of action

Studies suggest that some of the biological effects of the PFCs are mediated through PPARs and because PFOA and PFOS both activate murine PPAR α , the role of PPAR α in PFOA and PFOS immunotoxicity has been investigated. However, the specific role of PPARs in PFC-induced immunotoxicity is still a matter of debate (DeWitt et al., 2009b). Some data suggest that PPAR α mediates many processes connected with the immune system in an indirect fashion, by modulating lipid levels and leading to hepatotoxicity and stress effects (Qazi et al., 2009). Yang and co-workers (2002a) compared the immunomodulating effects of PFOA in wild-type and PPAR α null mice and reported that reductions in spleen weight and cellularity, in thymus weight and cellularity, and in mitogen-induced lymphocyte proliferation caused by PFOA in wild-type mice were not observed in PPAR α null mice, indicating that PPAR α plays a major role in the immunomodulation caused by PFOA. In contrast, DeWitt et al. (in preparation) found that suppression of antigen-specific IgM antibody responses occurred in PPAR α null mice was equivalent to that of wild-type mice exposed to the same dose of PFOA. The difference between the Yang et al. (2002a) and the DeWitt et al. (in preparation) studies was based on the strain of mouse; Yang et al. (2002a) evaluated immune responses in null animals on an Sv129 background and used a C57BL/6 animal as the corresponding wild-type whereas DeWitt et al. (in preparation) used C57BL/6 animals for both the null and wild-type. Therefore, it appears as if the immunotoxicity of PFOA *may* depend on PPAR α null mice in some strains of mice and *may not* in others. The immunotoxicity of PFOS appears to be partially dependent upon PPAR α activation (Qazi et al., 2009): reductions in thymus weight and in total number of thymocytes were only partially attenuated in PPAR α -null animals. In vitro studies have demonstrated that PFOA is a more potent agonist of murine PPAR α than PFOS (Takacs and Abbott, 2007), which may explain the differences in the immune responses in vivo. Effects independent of PPAR α activation may have greater potential impact on humans exposed to PFCs as human hepatic PPAR α expression is only one-tenth that of rodents (Kennedy et al., 2004). Furthermore, the affinity of PFOA and PFOS for human or murine PPARs is quite different. In vitro studies conducted on Cos-1 cells transfected with mouse or human PPAR α , β/δ , or γ reporter plasmids by Takacs et al. (2007) demonstrated that PFOA (0.5–100 μ M) significantly increased mouse and human PPAR α and mouse PPAR β/δ activity. Whereas PFOS (1–250 μ M) significantly increased activation of mouse PPAR α and PPAR β/δ isoforms, no significant activation of mouse or human PPAR γ was observed with PFOA or PFOS. The mouse PPAR α appears to be more sensitive to PFCs than the human PPAR α , with PFOA having more activity than PFOS with both the mouse and human PPAR isoforms, further supporting the hypothesis of a different role for PPAR α in PFOA and PFOS toxicity.

Modulation of cell-signaling responses is likely to be involved in PFC-induced suppression of antibody production, as alterations in the numbers of relevant leukocyte populations have not been demonstrated (Peden-Adams et al. 2010; Fair et al. 2011). After 28 days of oral exposure to PFOS and an in vivo antigen challenge, IL-6 production in B cells stimulated with anti-CD40 or soluble CD154 in vitro was suppressed. In the same study, nuclear translocation of NF- κ B (p65) and AP-1 (c-Jun) in splenic nuclear extracts was not altered, suggesting a possible role for altered CD40-CD154 signaling in the B cell (Peden-Adams et al. 2010).

PFOA and PFOS also inhibited T-cell-independent IgM antibody responses (TIAR) in mice: suppression of TIAR was observed in C57BL/6 female mice given 1.88 mg/kg of PFOA in drinking water for 15 days (DeWitt, unpublished data), and Peden-Adams et al. (2008) reported that exposure to PFOS also suppressed TIAR in B6C3F1 mice. The suppression of both TDAR and TIAR suggest that B cells, macrophages, or both may be specific targets of PFCs. Although these studies begin to elucidate possible pathways altered in PFOS-induced antibody suppression, additional studies are needed to clarify the mechanism associated with this effect.

Considering the ability of PFCs to directly affect immune cells, using human immune cells exposed to several PFCs in vitro, we demonstrated that PFCs directly affected immune cell activation and reduced cytokine production (both pro- and anti-inflammatory) (Corsini et al., 2011; Corsini et al., 2012). As plasma levels of PFOA and PFOS ranging between 3.7–12000 ng/ml have been found in the human population, the concentrations (0.1–10 μ g/ml) used in our in vitro studies are likely to be relevant for the human situation (Calafat et al., 2006; Olsen et al., 2007; Rayne and Forest, 2009; Steenland et al., 2010). We also demonstrated a role for PPAR α in PFOA-induced inhibition of cytokine secretion in human cells exposed in vitro. In contrast, we showed that the inhibitory effect of PFCs on in vitro cytokine production by human leukocytes can also occur independently of PPAR α , and involves the inhibition of NF- κ B activation. All of the PFCs studied, including PFOA and PFOS, decreased LPS-induced phosphorylation of p65 and NF- κ B driven transcription. However, with the exception of PFOA, none of the PFCs tested was able to activate PPAR α in transiently transfected THP-1 cells, excluding a role for this receptor in the immunomodulation observed. Similar to our previous findings with PFOS, PFBS and PFDA prevented LPS-induced I- κ B degradation (Corsini et al 2011). All of the PFCs tested inhibited p65 phosphorylation and NF- κ B driven transcription; and while it appears that PFOS, PFBS and PFDA act upstream by inhibiting I- κ B degradation, PFOA, PFOSA and fluorotelomer do not interfere with LPS-induced I- κ B degradation, suggesting a downstream effect. The phosphorylation of p65 is regulated by both cell- and stimulus-dependent activating kinases. Ser276 phosphorylation has a crucial role in the interaction with and the engagement of the cofactor CBP/p300, being therefore important in order to establish gene activation (Saccani et al., 2002; Vermeulen et al., 2002, 2003). The phosphorylation of p65, which is required for optimal NF- κ B dependent gene transcription (Schmitz et al., 2001), appears to be an important target of PFC-induced immunotoxicity. Figure 1 depicts the complexity associated with PFC-induced immunomodulation. The toxicity and immunomodulation induced by exposure to PFCs may occur via multiple pathways, and the

relative contributions of these pathways and the role of PPAR α likely differ among PFCs and animal species and/or strains.

6. Risk of immunotoxicity

Regulatory interest in PFCs recently has increased because of their ubiquitous presence in the environment, biological and environmental persistence, and reported experimental animal toxicities. In Europe, the European Food Safety Authority established an acceptable daily intake (ADI) for mammals of 0.0015 mg/kg bw/day for PFOA and 0.00015 mg/kg bw/day for PFOS (EFSA, 2012).

Regarding immunotoxicity, the weight of evidence supports the conclusion that antibody production is an endpoint sensitive to modulation by PFCs both in animals and humans. In mice, the LOAL for suppression of antibody production for PFOA was 3.75 mg/kg/d (corresponding to a serum concentration of 7.4×10^4 ng/ml) when given to female C57BL/6 mice by drinking water for 15 days (DeWitt et al. 2008) and 0.00166 mg/kg/day (corresponding to a serum concentration of 91.5 ng/ml) in male B6C3F1 mice given PFOS by oral gavage for 28 days (Peden-Adams et al. 2008). The margin of exposure (MOE), which is the ratio of the NOAEL (no observed adverse effect level) to expected human exposure, calculated from these studies in relation to the general human population serum PFC concentrations is approximately 15,000 for PFOA and 0.5 to 10.8 for PFOS. For occupational exposures, the calculated MOEs are 12 for highly exposed PFOA workers and approximately 0.005 for workers highly exposed to PFOS. Although MOEs are not values of risks themselves, when an MOE is less than 100, it is suggestive of potential health risks to exposed populations (Faustman and Omenn 2001). The serum levels associated with PFOA-induced immune effects are higher than the concentrations reported for humans and wildlife, whereas for PFOS, some human and wildlife species have serum concentrations that exceed the threshold for PFOS-induced immune effects, suggesting a potential risk for altered disease resistance.

Very recently, Grandjean et al. (2013) used the results obtained from the Faroese birth cohort study to establish a benchmark dose using linear and logarithmic curves. Calculations relied on serum-PFC measurements at age 5 years, and serum concentrations of specific antibodies two years later as clinically relevant measures of immune functions. The associations that appeared the strongest were selected for the benchmark dose calculations. Benchmark dose levels were about 1.3 ng/ml serum for PFOS and 0.3 ng/ml for PFOA at a benchmark dose response of 5%, and 0.8 and 5 ng/ml at a benchmark dose response of 10%. These results are below the range of average serum concentrations reported for general population. Using animal data, calculation of benchmark dose levels resulted in serum concentrations of 23 μ g/ml and 35 μ g/ml for PFOA and PFOS, respectively (Butenhoff et al., 2004). Based on animal data, US EPA has issued provisional health advisories of 400 ng/l for PFOA and 200 ng/l for PFOS in drinking water (US EPA, 2009). A lower guidance limit of 40 ng/l has been determined for PFOA by the state of New Jersey (Post et al., 2009). The authors concluded that if results are converted to approximate exposure limits for drinking water, current limits appear to be several hundred folds too high, and should be reconsidered.

7. Conclusions

The body of evidence concerning the ability of PFCs to modulate the immune system has grown in the last decade, and several studies have reported serum concentrations in rodents at immune effect levels within the range of human and wildlife exposures, suggesting risk of immunosuppression in humans, especially in children following pre-natal exposure, and in wildlife (DeWitt et al., 2012). Dysregulation(s) of immune system homeostasis can lead to adverse changes in immune functions, increasing the susceptibility to infections and cancer, as well as favoring the development of autoimmune diseases.

Our understanding of the mechanisms underlying the immune effects of PFCs is incomplete, as is our knowledge of how chemical structure and PPAR activation impact immune system toxicity. Because humans are exposed to both endogenous and exogenous PPAR ligands, the total ligand contribution on receptor occupancy and activities and subsequent influence on physiological processes deserves more attention.

While the phase-out of PFOA and PFOS resulted in a decrease in blood concentrations of these compounds in humans in the last decade (Kato et al, 2011), the concentration of other PFCs in humans has, however, increased in the same time period. Further studies are warranted, particularly studies that include biomarkers of immune system function and different PFCs in current and/or increasing use.

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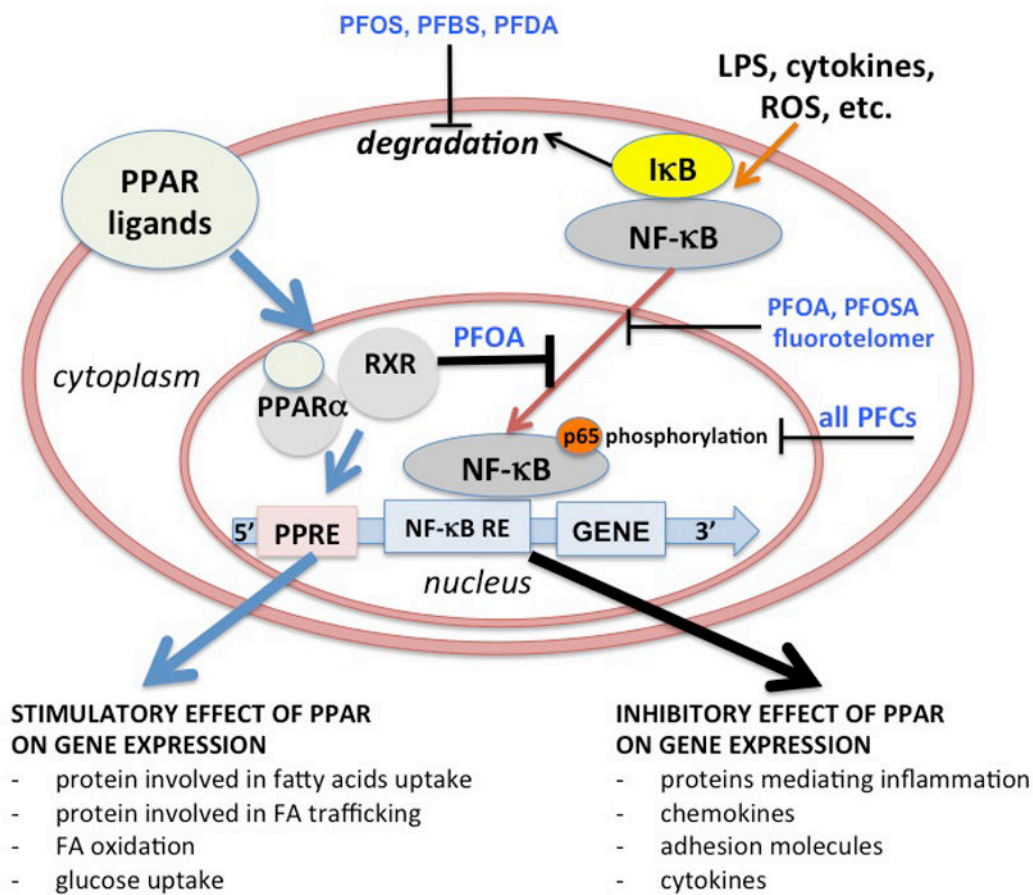


Figure 1. complexity associated with PFC-induced immunomodulation. The toxicity and immunomodulation induced by exposure to PFCs may occur via multiple pathways, and the relative contributions of these pathways and the role of PPAR α likely differ among PFCs and animal species. In human leukocytes, PFCs directly inhibit LPS-induced cytokine production. Both inflammatory cytokines as well as adaptive cytokines were affected by PFCs, with PFOA being the least effective. A role of PPAR α in PFOA-induced inhibition of cytokine secretion in human cells exposed in vitro was demonstrated. In contrast, the inhibitory effect of PFCs on in vitro cytokine production by human leukocytes occurred independently of PPAR α , and involves the inhibition of NF- κ B activation. In particular, PFOS, PFBS and PFDA act upstream by inhibiting I- κ B degradation, PFOA, PFOSA and fluorotelomer do not interfere with LPS-induced I- κ B degradation, suggesting a downstream effect. All PFCs tested inhibited the phosphorylation of p65 at Ser536, which is required for optimal NF- κ B dependent gene transcription and can explain the defective cytokine production following PFCs treatment (Corsini et al., 2012). PFBS, perfluorobutane sulfonic acid; PFDA, perfluorodecanoic acid; Fluorotelomer, 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptafluoro-(8:2 Telomer), RE, responsive elements.