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NEW INSIGHTS INTO PFAS IMMUNOTOXICITY

P. Grandjean[1], C. Heilmann[2], P. Weihe[3], E. Budtz-jørgensen[4]
[1]*University of Southern Denmark, Odense, Denmark*
[2]*National University Hospital, University of Copenhagen, Denmark*
[3]*Faroese Hospital System, Tórshavn, Faroe Islands*
[4]*University of Copenhagen, Denmark*

Introduction

While dioxin is a confirmed immunotoxicant, less documentation is available on such effects associated with other persistent pollutants. Recent data have documented that exposure to polychlorinated biphenyls (PCBs) is associated with immunotoxic effects. Among the outcomes studied, the immunological response to vaccinations has been verified as a feasible parameter and relevant parameter. The generation of specific antibodies directed against the antigen relies on major immune functions, and using the antibody concentration as the outcome is therefore appropriate and clinically relevant.

A brief background is appropriate (1,2). Upon the first encounter with a pathogen, a primary adaptive immune response is raised, where pathogen-specific naïve T and B lymphocyte clones are selected, expanded and differentiated into effector cells within the lymphoid tissue. Defense mechanisms differ according to the pathogen, the replication mechanisms of the microorganisms and the type of damage they cause. Generally, extracellular pathogens are accessible to soluble molecules, while intracellular pathogens are attacked by killing the infected cell. For extracellular pathogens, T cells activate pathogen-specific B cells and drive isotype switching and somatic hypermutation to produce plasma cells that produce high-affinity antibodies which are then transported in the blood to the site of infection. After successful clearance of the specific pathogen, the adaptive immune response results in a long-term protective immunity. The next encounter with a specific pathogen will provoke a faster and stronger response from long-lived memory B and T cells against the pathogen.

As routine childhood immunizations represent a standardized antigen provocation using a well-defined dose at particular ages, the response can be considered a natural experiment that can be utilized in epidemiological studies (3). While live (attenuated) vaccines are in common use, most studies of immunotoxicity have focused on inactivated vaccines, e.g., antigenic components of the pathogens, such as the purified exotoxins inactivated with formaldehyde as in the case of tetanus and diphtheria. The latter vaccines usually require the use of adjuvants and repeated inoculations and boosters to achieve the needed level of protection against microorganisms.

The animal data (4) as well as in vitro studies of human blood cells (5) have amply demonstrated that several perfluorinated alkylate substances (PFASs) are toxic to the immune system at serum concentrations that are similar to those that have been reported in occupational health studies or from the general population.

Materials and methods

We chose to generate birth cohorts for prospective studies in the Faroe Islands, a North Atlantic fishing community where exposures to methylmercury, PFASs, PCBs, and related contaminants cover a wide range. In addition to large-scale fishing, the Faroese conduct occasional subsistence whaling from small boats when pilot whale pods approach the coasts, and the meat and blubber are shared locally. While elevated concentrations of persistent halogenated pollutants occur in the pilot whales, the local differences in food preferences and the availability of whale result in widely different exposure levels.

The Faroese population of about 48,000 inhabitants resides on a dozen islands located between Shetland and Iceland. The Faroese are of Nordic and Irish origin and comparable in many ways to other Western populations. In this tightly knit society with minimal social differences, participation in prospective population studies is higher than in most other communities.

Prenatal exposure to contaminants is determined from pollutant concentrations in maternal blood during pregnancy or shortly after childbirth, or from analyses of cord blood. Clinical examinations at relevant ages provide an opportunity to collect interview information, blood samples for analyses, and results from physical examination and clinical tests. In accordance with international guidelines for evaluation of immune-related health outcomes (6), we examined children before and after the booster vaccination given at age 5 years.

Results and discussion

We first studied a birth cohort of 587 consecutive singleton births, with the first follow-up at ages 5 and 7 years (7). All participating children were vaccinated according to the official Faroese vaccination program, which includes vaccinations against diphtheria and tetanus at age 3, 5 and 12 months and a booster vaccination at age 5 years. Exposure to PFASs was assessed from analyses of maternal pregnancy serum and the child's serum at age 5 before the booster vaccination. Antibody concentrations were measured at age 5 years before the booster, approximately 4 weeks after the booster and again about 2.5 years later. Among PFASs in maternal pregnancy serum, perfluorooctanoic sulfonic acid (PFOS) showed the strongest inverse correlations with antibody concentrations at age 5 years. A doubled concentration of the three major PFASs in child serum at age 5 years was associated with a halving of the antibody concentration. Furthermore, a doubling of the serum concentrations of PFOS and perfluorooctanoic acid (PFOA) at age 5 was associated with odds ratios of 2.4-4.2 for having antibody concentrations below the clinically protective level of 0.1 IU/mL at age 7.

We have now extended the follow-up of this cohort to age 13 years (8). The serum-PFAS concentrations, especially for PFOS, had decreased during the six years since the previous examination, but some PFASs had increased. The two vaccine antibodies generally declined, most clearly for diphtheria. Records showed that 68 (13%) of the cohort members had visited the emergency room and likely received a vaccination booster. In addition, contrary to expectation, more than 200 children (39%) showed higher vaccine antibody concentrations at age 13 than at age 7, perhaps due to booster vaccinations. Thus, during the preceding six years, changes had happened that were not part of our epidemiologic protocol. Nonetheless, particularly in adolescents without a known booster, the diphtheria antibody concentrations tended to decrease at elevated PFAS concentrations at ages 13 and 7 years. Structural equation models showed that a doubling in PFAS exposure at age 7 was associated with losses in diphtheria antibody concentrations at age 13 of 10-30% for the five major PFASs. However, few associations were observed for anti-tetanus concentrations.

In parallel, we have recruited and examined a younger Faroese cohort, and the results at age 5 years concur with the findings in the older cohort and provide greater precision in regard to the dose-associated responses.

Based on the data from the older cohort, calculations of benchmark doses have been conducted (9). They rely on the serum-PFAS measurements at age 5 and the serum concentrations of specific antibodies two years later. Under different linear assumptions regarding dose-dependence of the effects, benchmark dose levels were about 0.13 ng/mL serum for PFOS and 0.03 ng/mL serum for PFOA. These results are below most serum concentrations reported in recent population studies and suggest that most current exposures may not be safe. When converted to approximate exposure limits for drinking water, current limits seem to be 100-fold too high.

Similar findings have been reported from Norway, where prenatal PFC-exposure and antibody responses to childhood vaccinations at age 3 years were analyzed in 56 children (10). Increased concentrations of several PFASs were significantly associated with reduced levels of anti-rubella antibodies in the children at age 3 years, although no significant associations in this small study were found with antibody responses to other vaccines studied.

In adults, the humoral immune response to seasonal influenza vaccination showed a reduced antibody response three weeks after immunization with an influenza strain at higher serum-PFOA concentrations (11). However, PFOS did not show any clear associations, an another flu strain did not reveal an effect, perhaps because the antibody responses involved a mixture of primary and secondary reactions as all participants had titers for some of these common viruses prior to the immunization.

The impact of immune dysfunction can be elucidated from the frequency of childhood infections. Thus, the Norwegian study showed a positive association between prenatal PFAS exposure and the self-reported number of episodes of common cold and gastroenteritis, although not for middle ear infection, during the first three years of life. We have extended these observations the Odense Child Cohort (Denmark), where maternal serum-PFAS concentrations were measured in early pregnancy. A total of 359 mothers reported on symptoms of infection in their child every two weeks for a one-year period. After adjustment for confounders and taking into account incomplete reporting, elevated PFOS exposure was associated with a significantly increased proportion of days with fever, while other symptoms, such as runny nose and cough, did not reveal any clear associations (12). Although signs of common infections may be a less sensitive indicators of immune system dysfunction than immunization responses, increased infection prevalence has important social implications.

Conclusions

Vaccination efficacy is a highly relevant parameter for assessment of immune suppression associated with environmental exposure to persistent halogenated pollutants. Results arising from vaccination

studies in children support the hypothesis that environmental exposure to PFASs may lead to compromised immune functions. An attenuation of vaccination responses may endanger the intended disease prevention of this public health effort, as illustrated by non-protective antibody concentrations in highly exposed children despite having completed all recommended vaccinations. Further, immune modulation may result in decreased resistance against infections in general, as has been found in studies of infants and small children. While some PFASs may be considered carcinogenic, the role of the immune system in this regard is so far unclear. Likewise, studies on allergy development and autoimmunity are needed to elucidate the wider implications of PFAS-associated immune dysfunction.

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