Autoantibodies Associated with Prenatal and Childhood Exposure to Environmental Chemicals in Faroese Children

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ABSTRACT

Methylmercury, polychlorinated biphenyls (PCBs), and perfluorinated compounds (PFCs) are ubiquitous and persistent environmental chemicals with known or suspected toxic effects on the nervous system and the immune system. Animal studies have shown that tissue damage can elicit production of autoantibodies. However, it is not known if autoantibodies similarly will be generated and detectable in humans following toxicant exposures. Therefore, we conducted a pilot study to investigate if autoantibodies specific for neural and non-neural antigens could be detected in children at age 7 years who have been exposed to environmental chemicals. Both prenatal and age-7 exposures to mercury, PCBs, and PFCs were measured in 38 children in the Faroe Islands who were exposed to widely different levels of these chemicals due to their seafood-based diet. Concentrations of IgM and IgG autoantibodies specific to both neural (neurofilaments, cholineacetyltransferase, astrocyte glial fibrillary acidic protein, and myelin basic protein) and non-neural (actin, desmin, and keratin) antigens were measured and the associations of these autoantibody concentrations with chemical exposures were assessed using linear regression. Age-7 blood-mercury concentrations were positively associated with titers of multiple neural- and non-neural-specific antibodies, mostly of the IgM isotype. Additionally, prenatal blood-mercury and -PCBs were negatively associated with anti-keratin IgG and prenatal PFOS was negatively associated with anti-actin IgG. These exploratory findings demonstrate that autoantibodies can be detected in the peripheral blood following exposure to environmental chemicals. The unexpected association of exposures with antibodies specific for non-neural antigens suggests that these chemicals may have toxicities that have not yet been recognized.

Key words: autoantibodies; mercury; polychlorinated biphenyls; perfluorinated compounds; prenatal; childhood; neurotoxicity; immunotoxicity

Methylmercury, polychlorinated biphenyls (PCBs), and perfluorinated compounds (PFCs) are environmental chemicals that are toxic to both the nervous system and the immune system (Crinnion, 2011; DeWitt *et al.*, 2012; Hong *et al.*, 2012; Post *et al.*, 2012). Methylmercury is primarily known as a neurotoxicant (Hong *et al.*, 2012), but it also is known to accumulate in numerous non-neural tissues (Nielsen *et al.*, 1994; Rodrigues *et al.*, 2010; Usuki *et al.*, 1998). PCBs, which are also known to be neurotoxic (Crinnion, 2011), may affect many different cell types through interaction with their cellular receptors (aryl hydrocarbon receptor and ryanodine receptors), which are broadly expressed (Gasiewicz and Rucci, 1984; Igarashi *et al.*, 1999; Lanner *et al.*, 2010; Mason and Okey, 1982; Quintana *et al.*, 2008) and regulate numerous transcriptional processes (Denison *et al.*, 2011). PFCs are thought to be neurotoxic as well (Gump *et al.*, 2011; Mariussen, 2012; Slotkin *et al.*, 2008), although their main effect may be immunotoxicity (Dong *et al.*, 2013; Grandjean *et al.*, 2012; Granum *et al.*, 2013). The diverse toxicity potentials and potential for broad transcriptional regulatory activity of these chemicals suggest that they may have toxicities beyond what has been identified thus far.

Exposure to such toxicants may leave behind evidence, in the form of long-lived antibodies specific to self-antigens derived from damaged tissues. Autoantibodies have been detected in the blood following damage to tissues in the periphery, such as damage to heart tissue following intraoperative myocardial ischemia (Bledzhyants *et al.*, 2007) and liver damage during hepatitis B infection (McFarlane *et al.*, 1995). Furthermore, autoantibodies have been detected in the peripheral blood after damage to brain tissue including following stroke (Bornstein *et al.*, 2001), head trauma (Marchi *et al.*, 2013), and neurodegenerative diseases (Diamond *et al.*, 2013), as well as following exposure to neurotoxic chemicals (Abou-Donia *et al.*, 2013; El-Fawal *et al.*, 1996; El-Fawal and McCain, 2008; El-Fawal and O'Callaghan, 2008). These findings suggest that autoantibodies may be used as surrogate measures of the occurrence (state), and possibly the severity (stage), of tissue damage from chemical exposure.

Because methylmercury has well-established neurotoxic effects (Hong *et al.*, 2012), exposure to this chemical might be expected to be associated with development of autoantibodies specific for neural antigens. In fact, autoantibodies specific to neurofilaments, astrocyte glial fibrillary acidic protein (GFAP), and myelin basic protein (MBP) were found in the peripheral blood following methylmercury exposure in rats (El-Fawal *et al.*, 1996). However, this has not yet been demonstrated in humans. Additionally, recent evidence suggests a link between PCBs and autoimmune responses including anti-nuclear and anti-thyroid antibodies. However, the primary target, and thus autoantibody profile, may differ depending on the specific mixture of congeners, dose, and sex (Cebecauer *et al.*, 2009; Gallagher *et al.*, 2013; Gu *et al.*, 2009). Therefore, we conducted a pilot study to assess the ability of autoantibodies to serve as biomarkers of past and present exposures to environmental chemicals.

MATERIALS AND METHODS

Subjects. A selection of 38 samples was made from a birth cohort established between 1986 and 1987 as part of the Children's Health and the Environment in the Faroes (CHEF) project at the National Hospital in Tórshavn, Faroe Islands. This cohort has been described previously (Grandjean and Weihe, 1993). The sample selection was constrained by the availability of sufficient serum from children who donated a blood sample at age 7. Ad-

ditionally, the selection aimed at providing a wide coverage of exposure levels.

Exposure measurements. As markers of methylmercury exposure, the total mercury concentrations were measured in cord blood and maternal hair as well as in the child's blood and hair at age 7 using cold-vapor atomic absorption spectroscopy (Grandjean and Weihe, 1993). Prenatal exposures to the PFCs perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) were assessed from analyses of cord blood; postnatal exposures were assessed from serum samples obtained from the child at approximately age 7. Analysis of coded samples for PFC concentrations was carried out by online solid-phase extraction and analysis using high-pressure liquid chromatography with tandem mass spectrometry (Haug *et al.*, 2009). PCBs were measured in cord blood and from the child's serum at age 7. We used a simplified ΣPCB concentration calculated as the sum of congeners CB-138, CB-153, and CB-180 multiplied by 2 (Grandjean *et al.*, 1995). Because the prenatal and age-7 exposures were measured in different media (e.g., prenatal PCBs in cord blood vs. age-7 PCBs in serum lipid), comparison of exposure levels between these time points requires consideration of conversion factors. However, we chose to use the analytical data without any conversions for the purposes of the present study.

Antibody measurements. Serum levels of autoantibodies to neurotypic and gliotypic proteins were determined as previously described (El-Fawal *et al.*, 1996; El-Fawal and O'Callaghan, 2008). Purified human proteins NF-L, NF-M, and NF-H, GFAP, actin, keratin, and desmin (American Research Products, Belmont, MA), choline acetyltransferase (ChAT) and MBP (Sigma-Aldrich, St. Louis, MO) were prepared in a 10 mM Tris-buffered saline (pH 7.4) at a concentration of 5 μg/ml. MBP was used at a concentration of 25 μg/ml. The choice of antigens was aimed at providing a representation of the cellular heterogeneity of the nervous system, secondary targets (i.e., muscle) and the more ubiquitous actin. Keratin was selected based on published reports of anti-keratin antibodies in hepatopathology and atopic dermatitis. Flat-bottomed Immulon microtiter plates (Fisher Scientific) were coated with 100 μl of the protein solution and incubated overnight at 4°C. The plates were washed three times with 10 mM Tris containing 0.05% Tween-80. Non-specific binding sites were blocked for 30 min at room temperature with 0.5% skim milk solution prepared in the Tris Buffer. Serum dilutions of 1:100 were prepared in the skim milk solution and 100 μl added per well, in triplicate. Plates were incubated for 2 h at room temperature and then washed three times with skim milk solutions containing 0.05% Tween-80. Alkaline phosphate goat anti-human IgG or IgM (Jackson Immunoresearch, West Grover, PA), at a 1:3000 dilution, was added to each well and the plates were incubated for 1 h. Following three washes with skim milk-Tween-80 solution and two washes with 10 mM Tris, the alkaline phosphatase substrate (p-nitrophenyl-phosphate, Bio-Rad, Richmond, CA) was added. The reaction was stopped by the addition of 100 μl 0.4 N NaOH per well. Plates were read at 405 nm in a plate reader (Tecan Spectrafluor). Generation of the standard curve for calculation of ng/ml.equivalence (ng/ml.eq) was performed using commercially available mouse monoclonal antibodies against these neuroantigens (American Research Products). The range of the 8-point standard curve was 0–2000 ng/ml and the limit of detection (LOD) for the assay was 0.002 ng/ml. All values from samples obtained from this cohort fell above the LOD and below the highest value in the standard curve.

Statistical analyses. All analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC). Differences between concentrations of IgM and IgG autoantibodies were assessed using a paired t-test. Linear regressions were performed to assess the associations of autoantibody levels with chemical exposures. Autoantibody values were natural log (ln)-transformed in order to obtain normally distributed residuals. Chemical exposure values were ln-transformed to allow the expression of the estimates as the percent change in autoantibody titer per two-fold increase in exposure. Residuals of the linear regression models indicated that the relationship between ln-transformed chemical values and ln-transformed antibody values were approximately linear and therefore that linear regression was an appropriate model for these data. For graphs in which autoantibody titers are displayed within tertiles of chemical exposure, cut points for each exposure were chosen so that each tertile contained approximately the same number of observations.

The mother's hair-mercury and child's hair-mercury were highly correlated with cord blood-mercury and age-7 blood-mercury, respectively, and therefore did not provide additional information to the model. The PFCs measured included perfluorohexane sulfonate and perfluorononanoic acid, but the values, especially in cord blood, were very low and were therefore disregarded. Child's sex was also assessed as a covariate in all models; however, adjustment for this did not change the association between the exposures and the autoantibodies and therefore was not included in the final models.

RESULTS

Among the 38 children whose samples were used in the present analyses, there were 16 males and 22 females with a mean age of 6.6 years at the time of sampling for autoantibody measurements and childhood exposures. The distributions of each of the prenatal and age-7 exposures to mercury, PCBs, and PFCs are shown in Table 1. A few of these exposures were moderately positively correlated with each other (Supplementary table 1).

The distributions of the titers of each of the autoantibodies are shown in Table 2. The concentrations of IgG were higher than those of IgM specific for the same antigen for all neural antigens. However, IgG was lower than IgM for all of the non-neural antigens (actin, desmin, and keratin). Many of the autoantibodies were moderately to highly positively correlated with several other autoantibodies (Supplementary table 2).

The association of each chemical exposure was individually assessed with each autoantibody titer using linear regression and the estimates for these associations (expressed as the percent change in antibody concentration per two-fold increase in exposure) are shown in Supplementary table 3. Age-7 mercury showed significant positive associations with multiple autoantibodies. These associations were almost exclusively with IgM and included the neural antigens NF-L (53.1% increase per two-fold increase in age-7 mercury), NF-H (40.0% increase), GFAP (30.4% increase), and the non-neural antigens actin (18.7% increase) and desmin (40.1% increase). NF-M-specific IgG was also significantly positively associated with age-7 mercury, showing an estimated 30.0% increase per two-fold increase in age-7 mercury. The antibody concentrations within tertiles of age-7 mercury are shown in Figure 1 and illustrate the dose-dependent increase in these autoantibody concentrations with increasing age-7 mercury concentrations.

Prenatal mercury and prenatal PCB exposures were found to be strongly negatively associated with the IgG isotype specific

solely for the keratin antigen (Fig. 2 and Supplementary table 3). Estimates indicate that anti-keratin IgG antibodies decreased by 59.9% and 77.3% with a two-fold increase in prenatal-mercury and prenatal-PCBs, respectively. When prenatal-mercury and prenatal-PCBs were included in the same model, the estimates for the strength of their association with anti-keratin IgG were modestly attenuated, although they remained significant, resulting in an estimated 46.8% and 67.0% decrease in autoantibodies with two-fold increases in prenatal-mercury and prenatal-PCBs, respectively (results not shown).

Age-7 PCBs were not found to be associated with any of the autoantibodies in the univariate models (Supplementary table 3). Additionally, PFOA was not found to be associated with any of the autoantibodies, at either the prenatal or the age-7 time point. However, although age-7 PFOS was not associated with any of the autoantibodies, prenatal PFOS was negatively associated with one autoantibody, actin-specific IgG, with an estimated 22% decrease in this autoantibody with a two-fold increase in prenatal PFOS exposure.

As PCBs and PFCs have known suppressive effects on antibody levels, suppression of the concentrations of autoantibodies due to concomitant exposure to PFCs or PCBs was considered. Therefore, PCBs and PFCs were evaluated as covariates in the models of the association of age-7 blood-mercury with autoantibodies. However, adjustment for neither PFOA nor PFOS, measured either prenatally or at age 7, nor PCBs measured at age 7, changed the estimates of the effects of age-7 mercury on autoantibody titers (results not shown).

Finally, evaluation of the effect of including prenatal mercury and age-7 mercury in the same model was conducted. In this model, the strength of the estimates of the association of age-7-mercury with autoantibodies modestly increased and the p-values decreased, resulting in the associations with ChAT-specific IgM and MBP-specific IgG to become significant (results not shown). However, this did not change the interpretation of the associations.

DISCUSSION

Autoantibodies have been suggested to be potentially useful for detection of tissue damage following chemical exposure (El-Fawal *et al.*, 1999; Evans, 1995). Therefore, the present study investigated the relationship between prenatal and childhood exposures to pervasive environmental chemicals and the development of antibodies specific for self-antigens. Despite our small sample size, we were able to detect substantial associations between prenatal mercury, prenatal PCBs, and childhood mercury exposure with the autoantibody titers. This has not been previously demonstrated in humans. Additionally, the associations with non-neural antigen-specific autoantibodies suggest that these chemicals may have toxicities on tissues not previously recognized. These findings demonstrate that autoantibodies can be detected following exposures to environmental chemicals and suggest that such antibodies may be useful biomarkers of tissue damage.

Interestingly, most of the autoantibodies that showed an association with age-7 blood-mercury were of the IgM isotype rather than IgG. This suggests that the tissue damage and the release of self-antigens may have occurred relatively recently or is on-going. Indeed, following exposure of rats to the neurotoxic chemical trimethyltin, IgM isotype autoantibodies were detected at the highest concentrations at week 1 following exposure compared with week 2 and 3, whereas the IgG isotype

TABLE 1. Distributions of Exposure Levels

Exposure	Age	Unit	N	Geometric mean (IQR[a])
Mercury	Prenatal	μg/l cord blood	38	31 (11–64)
Mercury	Age 7	μg/l whole blood	37	10 (5.5–23)
PCB	Prenatal	μg/l cord blood	37	2.0 (1.3–2.9)
PCB	Age 7	μg/g serum lipid	33	1.6 (0.97–2.9)
PFOA	Prenatal	μg/l cord blood	37	0.68 (0.41–0.98)
PFOA	Age 7	μg/l serum	34	4.3 (3.4–5.8)
PFOS	Prenatal	μg/l cord blood	37	3.1 (2.5–3.9)
PFOS	Age 7	μg/l serum	34	27 (22–35)

[a]IQR, interquartile range.

TABLE 2. Distributions of Autoantibody Concentrations (μg/l) (N = 38)

Antigen	Isotype	Geometric mean (IQR[a])
NF-L**	IgM	90 (29–245)
	IgG	3171 (1930–4731)
NF-M**	IgM	42 (30–57)
	IgG	91 (43–143)
NF-H*	IgM	151 (69–254)
	IgG	349 (111–1063)
GFAP**	IgM	103 (48–213)
	IgG	713 (605–837)
MBP**	IgM	93 (35–234)
	IgG	908 (552–1705)
ChAT**	IgM	2.2 (0.7–5.8)
	IgG	84 (57–124)
Actin**	IgM	926 (655–1300)
	IgG	581 (413–708)
Desmin	IgM	1.2 (0.46–2.4)
	IgG	1.1 (0.27–1.6)
Keratin**	IgM	1338 (824–3265)
	IgG	88 (7.6–947)

[a]IQR, interquartile range.
Difference between IgM and IgG nominal *$p < 0.001$, **$p < 0.0001$.

autoantibodies started to increase at week 2 and were the highest at week 3 (El-Fawal and O'Callaghan, 2008). It should also be pointed out that this paradigm is based on experimental models and evidence from vaccination studies. In chronic exposures, such as in the present study, IgG titers may indicate the initial or historical insult, whereas IgM and its association with age-7 mercury suggests an ongoing insult. For example, although both IgG and IgM isotypes are detected in multiple sclerosis patients, the latter isotype, against gangliosides, correlated with neurological disability (Vyshkina and Kalman, 2008).

The mechanism(s) through which mercury acts to produce the observed combination of associations with both neural and non-neural antigen-specific antibodies is not clear. All of the autoantibodies associated with age-7 mercury exposure may be related to each other through methylmercury's known neurotoxic effects. Methylmercury travels from the blood through the blood brain barrier and into to the brain (Aschner and Clarkson, 1988) where it can cause apoptotic cell death of neurons (Nagashima *et al.*, 1996), releasing neural antigens. Apoptotic cells are thought to be capable of eliciting autoantibodies (Navratil *et al.*, 2006) and this phenomenon may explain the association of neural antigen-specific antibodies with mercury in the present study. Additionally, although actin is not a protein specific to neural tissue, it is ubiquitously expressed, including in neural tissue (Matus *et al.*, 1982). Therefore, apoptosis of nerve

cells could release actin antigens and stimulate actin-specific autoantibody production in the same manner. Desmin, on the other hand, is not expressed in neural tissue—it is specific to muscle cells (Paulin and Li, 2004). It is conceivable that muscle atrophy secondary to peripheral nerve damage may release desmin antigens and result in generation of desmin-specific antibodies. Alternatively, accumulation of mercury in muscle tissues (Nielsen *et al.*, 1994; Usuki *et al.*, 1998) may directly induce apoptosis of muscle cells and release the desmin antigen that may then induce desmin-specific antibody production.

The present study did not find an association of neural antigen-specific autoantibodies with prenatal mercury exposure despite the fact that prenatal methylmercury exposure is known to cause substantial and diffuse neurotoxic effects. It is unclear why this was not reflected by an association of autoantibodies with prenatal mercury exposure as it was for childhood exposure. A possible explanation relates to the incomplete development of the adaptive immune system early in life. Although fetuses and newborns have the capacity to produce antibodies, the magnitude and durability of those antibodies are substantially limited by the immaturity of the immune system during these developmental periods (Siegrist and Aspinall, 2009). Therefore, it is possible that prenatal mercury exposure caused tissue damage that resulted in development of autoantibodies, but that those antibodies did not persist through age 7, resulting in the
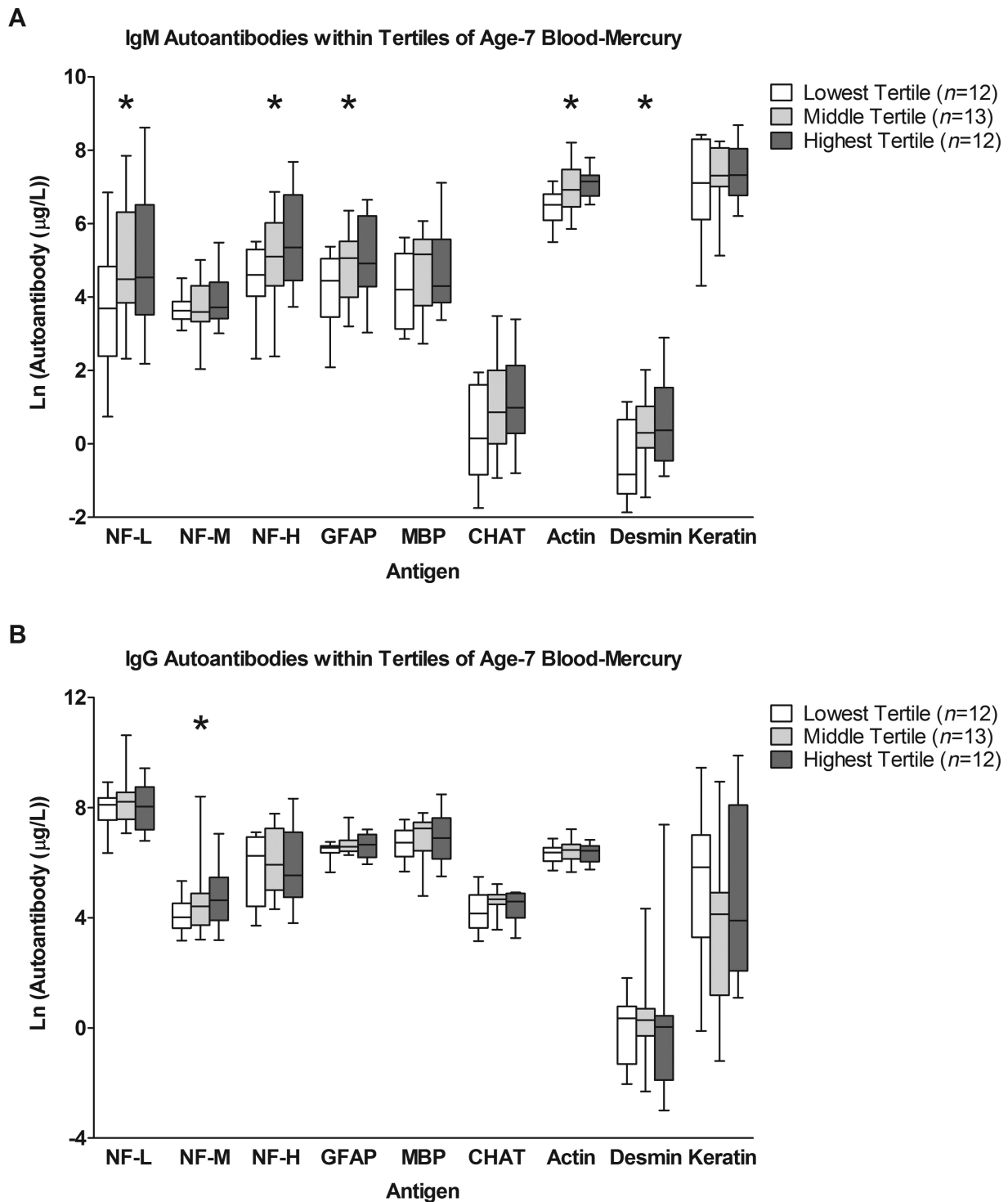
FIG. 1. Autoantibody titers within tertiles of age-7 blood-mercury. Age-7 blood-mercury values were separated into three groups of low, middle, and high values. For each antigen, distributions of autoantibody titers within each tertile are shown for IgM (A) and IgG (B) isotypes. Box plots show medians, interquartile ranges, and 95% confidence intervals. * indicate that the association between age-7 blood-mercury exposure and autoantibody titer for that antigen was significant based on linear regression analysis (p-value < 0.05).

lack of association of prenatal mercury with autoantibodies at age 7. Alternatively, it is plausible that IgG titers, which were higher than IgM, indicate successful development of immunological memory to earlier antigenic challenges occurring perinatally and in early childhood but that the dose response between

exposure and autoantibody was lost over time, whereas IgM reflects current status at the time of sampling at age 7. Finally, the nature of the toxicity of mercury on the nervous system may differ by stage of development such that antibodies are elicited by childhood exposure but not by prenatal exposure.
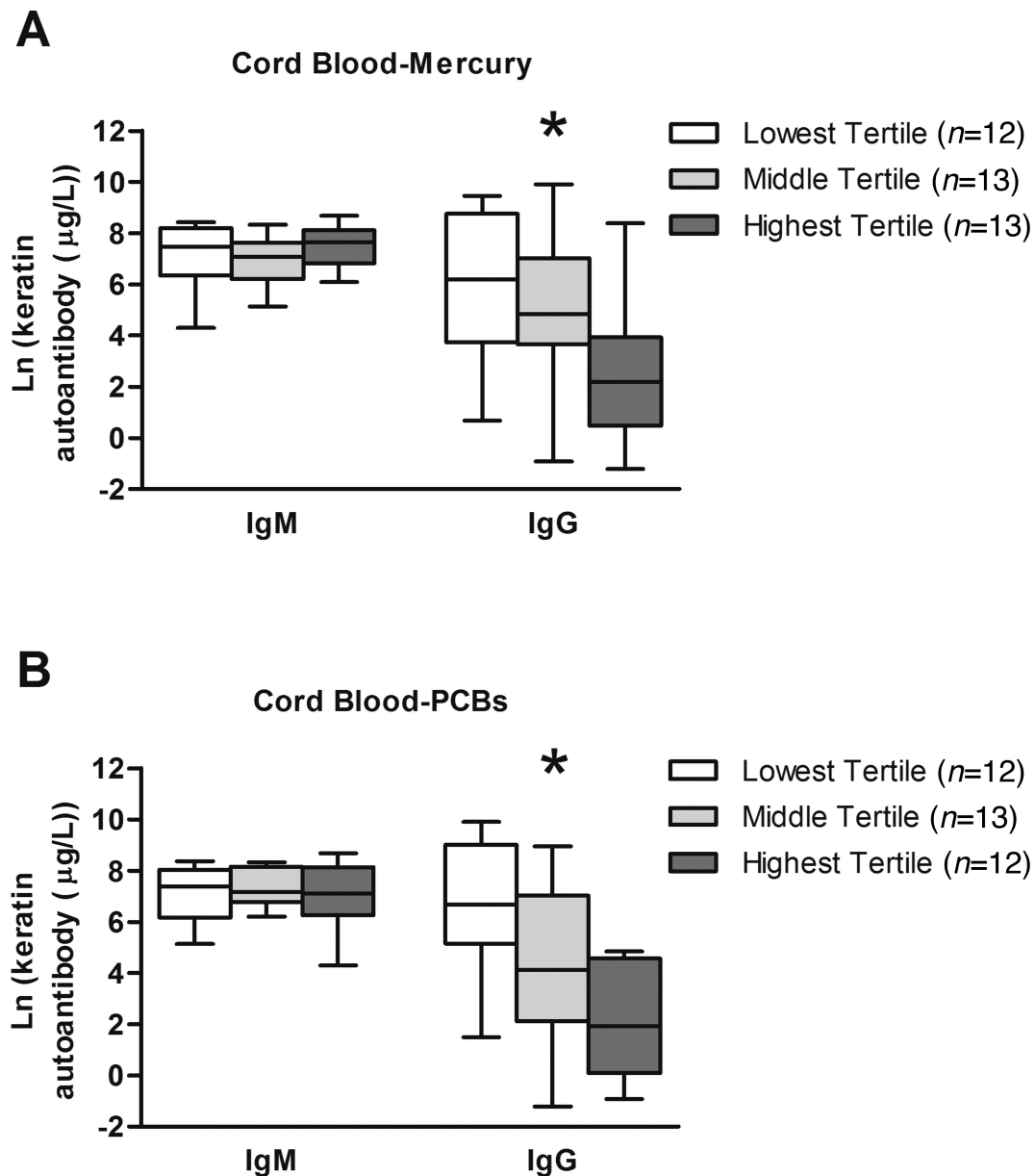
A
Cord Blood-Mercury

B
Cord Blood-PCBs

FIG. 2. Keratin-specific autoantibody titers within tertiles of cord blood-mercury and cord blood-PCBs. Cord blood-mercury and -PCB values were separated into three groups of low, middle, and high values. Distributions of the IgM and IgG keratin-specific autoantibody titers within each tertile of cord blood-mercury (A) and cord blood-PCBs (B) are shown. Box plots show medians, interquartile ranges, and 95% confidence intervals. * indicate that the association between age-7 blood-mercury exposure and autoantibody titer for that antigen was significant based on linear regression analysis (p-value < 0.05).

Associations of prenatal mercury and prenatal PCBs with IgG antibodies specific for the non-neural antigen keratin were also found. Given the multiple comparisons that were performed in the present analysis, these associations may be a result of random variation. However, the fact that these prenatal exposures were associated with autoantibodies of the IgG isotype is consistent with the insult occurring in the past, rather than recently. The inverse associations between these exposures and the keratin autoantibody titers were unexpected. This not only suggests that these chemicals may have an immunosuppressive effect, but also that this effect may occur only when exposed during early development. However, why this immunosuppressive effect was specific to keratin autoantibodies is unclear. It is interesting that we have previously reported that PCB exposure

was negatively associated with the presence of atopic dermatitis (Grandjean et al., 2010), a disease of the skin that has been associated with keratin-specific autoantibodies (Natter et al., 1998; Samoylikov et al., 2013). Assaying for autoantibodies specific to a larger panel of autoantigens, as well as other immunoglobulin isotypes, would help determine if this is reflective of a suppressive effect due to toxicity specifically targeted to a keratin-expressing cell type or of more global immunological dysregulation.

Finally, we found a general lack of associations of autoantibodies with PFC exposure. Although, we did find that prenatal PFOS was negatively associated with one autoantibody, anti-actin IgG. However, because actin is a ubiquitous protein, the specific tissue damage that may be associated with prenatal

PFOS exposure is unknown. It is also possible that the association with actin-specific antibodies was found by chance, especially because most of the other estimates of the associations with prenatal PFOS are in the opposite direction. A larger study should be conducted to determine if the association with anti-actin IgG can be replicated and also to confirm all other associations that were found is this study.

Certain PFCs are carcinogenic (Lau *et al.*, 2007), immunotoxic (Dong *et al.*, 2013; Grandjean *et al.*, 2012; Granum *et al.*, 2013), and may also be neurotoxic (Gump *et al.*, 2011; Mariussen, 2012; Slotkin *et al.*, 2008). Although the carcinogenicity associated with some PFCs has been attributed to the ability to act as peroxisome proliferator-activated receptor alpha (PPARα) agonists (Lindstrom *et al.*, 2011; Vanden Heuvel *et al.*, 2006), it is unclear if other toxicities are also PPARα-dependent (Harada and Koizumi, 2009). PPARα activation decreases expression of the transcription factor T-bet (Gocke *et al.*, 2009), and T-bet promotes antibody isotype switching in B cells and possibly autoantibody production (Peng *et al.*, 2002). Although suppression of T-bet expression by PPARα has been demonstrated only in T cells so far, similar effects in B cells could conceivably lead to protection from autoantibody production. However, the associations observed between PFCs and autoantibodies do not support this notion, which is in agreement with a recent study in mice that failed to identify associations of neural and non-neural autoantibody concentrations with PFOA exposure (Hu *et al.*, 2012).

The blood concentrations of the PFCs in these Faroese children were similar to those found in other countries at comparable time periods, including in the United States (Kato *et al.*, 2011). However, the concentrations of both mercury (CDC, 2004) and PCBs (Nichols *et al.*, 2007) were substantially higher in these Faroese children compared with many other countries, except for populations that also have high exposure to these chemicals due to their seafood-based diets, such as the Inuit (Llop *et al.*, 2012). This highlights the importance of determining if alterations in autoantibody titers also are seen in populations that experience exposures lower than these Faroese children.

The finding that all children had detectable levels of all autoantibodies evaluated is not surprising. It has been shown both in healthy newborns and in healthy adults that the human antibody repertoire contains thousands of antibodies specific to self antigens (Merbl *et al.*, 2007; Nagele *et al.*, 2013). However, many of the studies that have measured these natural autoantibodies have neither identified the specific antigens that the antibodies were binding nor were able to determine the concentrations of these autoantibodies. Therefore, normal ranges of concentrations are not known for most autoantibodies. Consequently, we were unable to identify children in the present study with autoantibody concentrations outside expected ranges. Such information would be crucial to facilitate any future implementation of autoantibodies as biomarkers of tissue damage associated with environmental chemical exposure.

The present study found that environmental chemical exposures were associated with concentrations of antibodies specific to both neural and non-neural antigens. Whether these autoantibodies are side events or perhaps pathogenic so that they are involved in subsequent development of autoimmune disease would require further investigation. Nevertheless, the detection of neural antigen-specific autoantibodies may be of particular interest because these antibodies may be used as accessible biomarkers of chemically induced damage that may lead to neurological delays, deficits, or diseases. Follow-up studies should be conducted on the larger cohort of children to assess if the presence and concentrations of these autoantibodies can predict the development, and possibly severity, of neurological and/or autoimmune outcomes. Finally, the associations with non-neural antigen-specific autoantibodies were unexpected and suggest that these chemicals may cause toxicities that have yet to be recognized. Future studies should confirm these associations with non-neural antigen-specific autoantibodies and evaluate the nature of the tissue damage that is causing them.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci. oxfordjournals.org/.

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