Expression of aryl hydrocarbon receptor, inflammatory cytokines, and incidence of rheumatoid arthritis in Vietnamese dioxin-exposed people

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ABSTRACT
Many Vietnamese citizens have been and continue to be inadvertently exposed to dioxins and dioxin-like compounds deposited in the country during the Vietnam War. Dioxins may be involved in the pathogenesis of inflammatory diseases in part via by affecting expression of aryl hydrocarbon receptor (Ahr) and inflammatory cytokines in animal models. As the role of the Ahr in dioxin-exposed people is not well defined, a study was conducted to examine gene expression levels of Ahr, inflammatory cytokines, and the incidence of diseases in dioxin-exposed citizens who had/still resided near a heavily dioxin-contaminated area in Vietnam. Whole blood from citizens at/around Da Nang airbase and control individuals living in unsprayed areas was collected. Serum levels of dioxins were analyzed by using a dioxins-responsive chemical-activated luciferase gene expression bioassay. Gene expression of Ahr, interleukin (IL)-1β, TNFα, IL-6, and IL-22 in whole blood was examined by quantitative real-time PCR. The results showed levels of dioxin and expression of Ahr, IL-1β, TNFα, and IL-6 were up-regulated while IL-22 expression was down-regulated in dioxin-exposed people. Various disease incidences in the study subjects was also examined. Interestingly, the incidence of rheumatoid arthritis (RA) in these individuals was increased compared to the estimated prevalence of this disease in the general Vietnamese population. Analyses also showed that expression levels of Ahr correlated to those of IL-6 and IL-22 in the dioxin-exposed people. Taken together, dioxins might be involved in an up-regulated expression of Ahr that might possibly relate to changes in level of inflammatory cytokines and, ultimately, in the incidence of select diseases in residents of Vietnam who had/continue to live near a dioxins-contaminated site.

Introduction
The term “dioxins” includes 75 PCDD (poly-chloro-dibenzo-p-dioxins) compounds, 135 PCDF (polychloro-dibenzo-furans), and 209 PCB (polychloro-biphenyls) compounds in which 2,3,7,8-tetrachlorodibenzop-p-dioxin (TCDD) is the most toxic compound (ATSDR 1998; Tuyet-Hanh et al. 2016). As a class of compounds, dioxins are highly toxic environmental contaminants that possibly cause a variety of health issues in exposed people, including disorders of the immune-, reproductive-, endocrine-, and nervous systems (Institute of Medicine 2014). Aryl hydrocarbon receptor (Ahr), a ligand-dependent transcription factor, has been shown to act as a binder of such ligands as TCDD, 6-formylindolo[3,2-b]-carbazole (FICZ), and kynurenine (KYN) (Nguyen and Bradfield 2008; Quintana et al. 2008; Veldhoen et al. 2008; Stevens et al. 2009; Mezrich et al. 2010; Denison et al. 2011; Wincent et al. 2012).

TCDD/other dioxins induce translocation of Ahr from the cytoplasm to nucleus where it forms a complex with the aryl hydrocarbon receptor nuclear translocator (ARNT) that, in turn, binds xenobiotic-responsive elements (XRE) in promoters of responsive genes, including members of the cytochrome P450 family (Perdew 1988; Burbach et al. 1992; Mimura et al. 1998; Kazlauskas et al. 1999). The Ahr has also been shown to play a pivotal role in immune system cells involved in development of inflammatory diseases in animal models of experimental autoimmune encephalomyelitis (EAE), dextran sulfate sodium-induced colitis (DSS-induced colitis), and collagen-induced arthritis (CIA). This is due, in part, to a role in regulation of production of inflammatory cytokines such as interleukin (IL)-1β, -6, and -17, and tumor necrosis factor (TNF)-α by macrophages, dendritic cells, and imbalanced T-cell differentiation into regulatory T (Treg) cells or IL-17-producing T-helper (Th17) cells (Kimura et al. 2008; Quintana et al. 2008; Veldhoen et al. 2008; Marshall and Kerkvliet 2010; Nguyen et al. 2010, 2013; Nakahama et al. 2011, 2013; Stockinger et al. 2011; Chinen et al. 2015).

People with high levels of exposure to dioxins often suffered from an array of diseases that were normally associated with alterations in host immunity (Tai et al. 2011; Nishijo et al. 2014; Suzuki et al. 2014). Rheumatoid arthritis (RA) is a chronic
inflammatory disease whose development and severity appear to be exacerbated by dioxin exposure (Kobayashi et al. 2008). A role for AhR in RA was suggested by Nakahama et al. (2011) who showed that AhR-deficient mice failed to develop CIA, an animal model of human RA (Nakahama et al. 2011). Given the importance of dioxins and disease pathogenesis, it was of interest to see if there might be a potential link among levels of dioxins, expression levels of AhR, inflammatory cytokines, and the incidence of diseases including RA in dioxin-exposed people. If this was the case, AhR-targeting therapies could then potentially be developed to improve the health of these afflicted individuals.

Da Nang airbase is considered as a hotspot for war-derived dioxins contamination in Vietnam (Stellman et al. 2003; Dwernychuk 2005; Tuyet-Hanh et al. 2015; Ngo et al. 2017). Because of the potential exposure to still-high level of dioxins by residents near this site, the aim of the current study was to investigate the levels of dioxins and the expression levels of AhR and inflammatory cytokines such as IL-1β, TNFα, IL-6, and IL-22 in the whole blood of dioxin-exposed Vietnamese people living near Da Nang airbase. As controls, age-matched individuals who lived in unsprayed areas in northern Vietnam were also evaluated.

Materials and methods

Subjects

This study was performed at the 103 Military Hospital located within the Vietnam Military Medical University, in accordance with the Declaration of Helsinki (World Medical Association 2013). A total of 60 dioxin-exposed people (36 females, mean age 52.5 yr [50.0–58.0], body mass index (BMI) = 21.8 [20.0–24.2], and 24 males, mean age 60.0 yr [55.2–65.7], BMI = 22.3 [20.9–23.7]) who had resided near Da Nang airbase for > 10 yr, and 20 healthy age- and BMI-matched people (both genders) who lived in northern Vietnam (controls), were recruited for this study. Subject length of time living near Da Nang airbase was obtained by interview. Health status was evaluated by general examination had occurred. Estimates of bioanalytic equivalents (BEQ)/g fat in each sample were then calculated as described in Khoa et al. (2015).

RNA isolation and reverse transcription

Total RNA was extracted from the whole blood with TriPure Isolation Reagent (Roche Diagnostics, Mannheim, Germany) according to the manufacturer’s instructions. RNA quality was confirmed with 260/280 nm ratios [1.8–2.0] obtained in a NanoDrop Lite spectrophotometer (ThermoFisher Scientific, Wilmington, DE). Complementary DNA (cDNA) was synthesized from 0.5 μg total RNA/sample. Synthesis of first-strand cDNA was performed using recombinant transcription reagents immediately following RNA isolation. Reverse transcription was then performed using a RevertAid First Strand cDNA Synthesis Kit (ThermoFisher Scientific, Waltham, MA). Each transcription was carried out in 5 × reaction buffer containing 10 mM dNTP mixture, 20 U RNase inhibitor/μl, 200 U M-MuLV reverse transcriptase/μl, random hexamer primer, 0.5 μg sample RNA, and RNase-free water (to a final volume of 20 μl). The reaction was incubated at 42 °C for 60 min, and the transcriptase then inactivated at 70 °C for 5 min. Control without reverse transcriptase was performed for each RNA sample to verify no DNA contamination had occurred. β-actin was used as an internal reference.

Quantitative real-time RT-PCR assay

For analysis of AhR, IL-1β, TNFα, IL-6, IL-22, and β-actin expression, respective cDNA samples were amplified and quantitative PCR performed using LightCycler FastStart DNA Master SYBR Green I and a Light Cycler 2.0 instrument (Roche Diagnostics). Each reaction contained the same volume sample template (cDNA), Light-Cycler FastStart Reaction Mix (5×), forward primer (0.5 μM), reverse primer (0.5 μM), and dH2O [to 20 μl]. The primers used are summarized in Table 1. Thermal cycling comprised a denaturation step at 95 °C for 10 min, followed by 40 cycles using 95 °C for 10 s, 60 °C for 10 s, and 72 °C for 20 s. At the end of the PCR, a melting curve was generated to verify product specificity. A non-template control was carried out in every assay; no PCR contamination was observed. Levels of each mRNA were calculated by the 2-ΔΔCT method (Livak and Schmittgen 2001). β-actin was used as an internal control. Each control value was set at 1.0 and was in turn used to calculate fold-change values.

Statistical analysis

All data are presented as median [interquartile range]. Statistical comparisons were performed using a Mann–Whitney U-test.
A Pearson’s rank correlation was used to analyze correlation among dioxin levels, and the gene expression levels of Ahr and inflammatory cytokines. A p values < 0.05 was considered statistically significant. Data were analyzed with SPSS software (SPSS, Chicago, IL).

Results

Dioxin levels

Dioxins (hereafter referring to TCDD and many other types in this class of compounds) remain contaminants in soil, water, and food around Da Nang airbase. And yet, data about dioxin levels in people in the area are still limited (Forrest et al. 2005; McHale et al. 2007). DR-CALUX analysis of serum from people who had/still reside near the airbase and controls living in unsprayed areas in northern Vietnam revealed a significant presence of dioxins in Da Nang subjects (Figure 1(A)). Levels of dioxins in these individuals were significantly higher vs. controls (62.03 BEQ/g fat [40.74–89.42] vs. 17.45 BEQ/g fat [12.05–35.57]) (p < 0.005).

Ahr expression

As many forms of dioxins are potential Ahr ligands, Ahr expression in whole blood obtained from the study subjects was assessed via quantitative real-time PCR. Figure 1(B) shows that Ahr expression was significant elevated 16.39-fold [8.25–28.26] (p < 0.05) in the blood of people that had/still resided near Da Nang airbase compared to in the blood of control subjects. There was no correlation between blood levels of dioxins and Ahr expression levels in those hosts (data not shown). Blood levels of dioxins and Ahr expression levels did not differ by gender (data not shown).

To determine whether the potential differences in Ahr expression might be associated with incidence of RA, the study’s dioxins-exposed individuals were separated into two groups. The results showed that Ahr expression in the blood of those with RA (n = 6) was increased (33.91-fold [13.64–67.07] vs. control) compare to that of those without RA (n = 54) (13.72-fold [8.57–26.71] vs. control) (Figure 2). These differences in expression were not significant.

Table 1. Sequence of primers used in real-time PCR, amplicon sizes, and annealing temperature.

| Target, Genbank accession # | Sequence | Amplicon length (bp) | Temperature (°C) |
|-----------------------------|----------|----------------------|-----------------|
| AhR, NM_001621              | F: 5’ ACATCACCTAGCCAGTCGC 3’ | 101               | 60              |
|                             | R: 5’ CTATGGGCGTTGGAAGGAT 3’  |                   |                 |
| IL-1β, NM_000576            | F: 5’ ACAGATGAAGTGCTCCTCA 3’  | 731               | 60              |
|                             | R: 5’ GTAAGATGGTCAGCTGAT 3’    |                   |                 |
| IL-6, NM_000600             | F: 5’ ACTACCTCTTCAGAAGAATTG 3’ | 149               | 60              |
|                             | R: 5’ CCATCTTGGAAAGTTCAAGTTG 3’|                   |                 |
| IL-22, NM_020525            | F: 5’ TGGTTGCTCCCTCAAATG 3’    | 86                | 60              |
|                             | R: 5’ TGCGTTAGCGCTTGCTG 3’     |                   |                 |
| TNFα, NM_000594             | F: 5’ ATGAGGACAAAGGACCAGTGC 3’ | 217               | 60              |
|                             | R: 5’ GAGGGCTGATTGAGAGAGGTC 3’ |                   |                 |
| β-actin, NM_001101          | F: 5’ TCATGAGAGTGCTGGAGACATC 3’| 156               | 60              |
|                             | R: 5’ CAGAGGAGCAATGATCCTTGACT 3’|                   |                 |

Figure 1. Host blood levels of dioxins and Ahr expression levels. (A) Dioxins in serum (***p < 0.005). (B) Ahr in whole blood (*p < 0.05). Boxes represent interquartile range (IQR), 25th and 75th percentiles, minimum and maximum values. Horizontal lines within boxes represent medians. Whiskers extend to 1.5-times the IQR. Circles represent outliers; star signs represent extreme outliers. Control = individuals living in unsprayed areas. Dioxin = dioxin-exposed people.
**Inflammatory cytokine gene expression**

Ahr plays a crucial role in many types of immune cells by impacting on the presence of inflammatory cytokines that, in turn, may drive development of inflammatory/autoimmune diseases. As shown in Figure 3, the expression level of IL-1β, TNFα, and IL-6 was increased while that of IL-22 was decreased in the dioxins-exposed people who had/still resided near Da Nang airbase compared to values in controls. The increased expression levels of IL-1β, TNFα, and IL-6 in dioxins-exposed subjects reflected significant \( p < 0.05 \) 11.18-fold [6.76–20.72], 6.64-fold [3.43–13.67], and 2.93-fold [1.80–5.18] changes, respectively, vs. control levels. The decreased expression of IL-22 in dioxins-exposed study subjects was 0.05-fold [0.01–0.11] \( p < 0.01 \) vs. controls. No correlation between blood levels of dioxins and IL-1β, TNFα, IL-6, and IL-22 expression levels was found (data not shown).

**Correlation between Ahr and inflammatory cytokine expression in dioxin-exposed people**

Ahr expression levels in whole blood samples were evaluated in the context of selected inflammatory cytokine expression levels in the study subjects to see if there was any correlation. Interestingly, a weak positive correlation was observed between Ahr expression and that for the genes for IL-6 \( (r = 0.287, p < 0.05) \) and IL-22 \( (r = 0.332, p = 0.01) \) (Figure 4). In comparison, there was no apparent correlation between the expression of Ahr and that of genes for IL-1β, TNFα, IL-6, and IL-22 expression levels was found (data not shown).

**Incidence of diseases in dioxin-exposed people**

Exposures to dioxins are known to possibly result in various diseases in humans (Institute of Medicine 2014). Table 2 illustrates findings showing that among the 60 people in this study who had/still resided near the airbase, several suffered a range of diseases, i.e. cardiac (18.3%), rheumatologic (16.7%), digestive (10.0%), endocrine (6.7%), neurologic (6.7%), reproduction (5.0%), dermatologic (5.0%), urologic (3.3%), respiratory (1.7%), and indeterminate (16.6%). As a specific pathology, RA was noted at a level of 10.0%, higher than the estimated RA prevalence rate (0.5%) in the general Vietnamese population (An and Khoa 1996). Notably, in Table 2, no patient had more than one category of affliction. It would have been interesting to discern...
any links to gender with each of the noted disease incidences. Though the data reflect how many subjects (M vs. F) had the particular pathology noted, unfortunately, statistical conclusions about M vs. F cannot be reached at this time. Follow-on studies will attempt to expand the population size captured (i.e. increase disease incidence levels) in order to permit such types of statistical analyses to be performed properly.

Discussion

Dioxins (including TCDD and several other agents in this class) have long half-lives in soils, ranging from decades in surface soil up to a [calculated] century in subsurface soils (Paustenbach et al. 1992; Durant et al. 2015). Da Nang airbase is considered one of two highly dioxins-contaminated areas in Vietnam. Consequently, residents living around this area remain at high risk of exposure to dioxins through contaminated environment even though more than 40 years has passed since the war.

Dioxins activate Ahr signaling in many types of cell in both animals and humans (Schultz et al. 2003; Esser et al. 2009; Vogel et al. 2014). Here, it was seen that both serum levels of dioxins and whole blood Ahr expression levels were significantly elevated among the dioxin-exposed people of this area (Figure 1). An important issue relevant to the dioxins measurements that underpin this whole study was residence time, i.e. whether all dioxin-exposed people still lived in the area from the start or they recently entered the area as well as how this might impact the measured outcomes of dioxin levels. While the current study collected blood from exposed people who had lived for a minimum of ≥10 year near the airbase, it did not segregate out populations based on cumulative potential years of exposure (i.e., 10–20, 20–30, 30–40, >40 years). While it would be a great asset to be able to perform such detailed “time-dependent” population (i.e., 10 vs. 20 vs. 30 yr exposure groups) analyses, the sample sizes would have to be vastly expanded to capture these groups and then age-match all the subjects for age at initial exposure, cumulative potential years of exposure, etc. Similarly, analyses of where each individual lived could be correlated to levels of dioxins in their habitats, and all the above-analyses re-visited. Again, while this would be valuable information to have, it would be impossible to attain as development of the areas around the base over the past >40 years have clearly made such potential analyses impossible for any specific home/local site for any given study subject.

The current study found, relative to in control subject samples, increased expression levels of genes for such pro-inflammatory cytokines as IL-1β, TNFα, and IL-6 in the dioxins-exposed individuals. Interestingly, IL-22 expression levels were reduced in these individuals. Potential effects from IL-1β, TNFα, and IL-6 over-expression with regard to inflammatory and/or autoimmune processes have been well-defined (O’Shea et al. 2002; Navegantes et al. 2017). Accordingly, neutralizing antibodies against IL-6 and TNFα seem promising as therapies against many inflammatory/autoimmune diseases (Tanaka and Kishimoto 2014; Sedger and McDermott 2014; Di Paolo and Shayakhmetov 2016).

IL-6 is known as a pleiotropic cytokine that acts on many types of cells, including T-cells, B-cells, and synovial fibroblasts, and also contributes to the pathogenesis of several autoimmune diseases including RA (Tanaka et al. 2014). The latter is borne out by findings of elevated levels of IL-6 in serum and joint (synovial) fluids isolated from RA patients (Hirano et al. 1998). Exact mechanisms for how IL-6 does contribute to RA remain to be fully defined but it is known that IL-6 is a factor in the process

| Diseases | Number of persons | Incidence (%) |
|----------|------------------|---------------|
| Cardiovascular | 11 (4 M, 7 F) | 18.3 |
| Rheumatology | 10 (5 M, 5 F) | 16.7 |
| Rheumatoid arthritis (RA) | 6 (1 M, 5 F) | 10.0 |
| Digestion | 6 (4 M, 2 F) | 10.0 |
| Endocrinology | 4 (2 M, 2 F) | 6.7 |
| Neurology | 4 (1 M, 3 F) | 6.7 |
| Reproduction | 3 (1 M, 2 F) | 5.0 |
| Dermatology | 3 (0 M, 3 F) | 5.0 |
| Urology | 2 (2 M, 0 F) | 3.3 |
| Respiratory system | 1 (0 M, 1 F) | 1.7 |
| Indeterminate | 10 (4 M, 6 F) | 16.6 |

No patient had more than one category of affliction. M: male; F: female.

Figure 4. Correlation between expression levels of Ahr, IL-6, and IL-22 in dioxins-exposed subjects. A weak positive correlation was observed between expression of Ahr and of IL-6 (r = 0.287, p < 0.05) and of Ahr and of IL-22 (r = 0.332, p = 0.01).
of naïve T-cell differentiation into Th17 cells (Bettelli et al. 2006; Acosta-Rodriguez et al. 2007) that are critical for autoimmune disease development (Afkali et al. 2007). It is worth noting that Ahr on naïve T-cells is induced by stimulation with transforming growth factor (TGF)-β plus IL-6 during Th17 cell differentiation (Kimura et al. 2008; Quintana et al. 2008; Veldhoen et al. 2008). It is interesting to speculate that if there is a positive correlation in expression patterns between Ahr and IL-6 in MCF-7 cells (Hollingshead et al. 2008; DiNatale et al. 2010; Kolasa et al. 2013), in TCDD-treated animals (Chmill et al. 2010) or in humans (Chen et al. 2012), and any potential IL-6-Ahr axis regulates Th17 cell generation. Then, neutralizing antibodies against IL-6 could be useful not only for the treatment of RA in general, but among dioxin-exposed people it could help short-circuit this axis and thus prevent many pathologies that arise from excessive Th17 cell presence in a host.

On the other hand, the impact of changes in IL-22 is more complicated in that IL-22 has a binary function in immune responses depending on context (Sanjabi et al. 2009). For example, IL-22 has an inflammatory role in different chronic inflammatory conditions, including psoriasis and dermal inflammation (Wolk et al. 2004; Zheng et al. 2007). Conversely, IL-22 can have a protective role in acute inflammation state as arise during hepatitis and inflammatory bowel disease (Zenevicz et al. 2007, 2008). Reduced IL-22 expression level in dioxin-exposed people was consistent with results showing reduced IL-22 levels in PCB-contaminated Yusho patients (Kuwatsuka et al. 2014). As various cell types can produce pro-inflammatory cytokines, further in vitro studies could reveal which types of immune system cells might be affecting the changes in inflammatory cytokine expression being noted in dioxin-exposed people.

Analyses here also revealed a weak positive correlation between Ahr expression level and IL-6 and IL-22 expression in the dioxins-exposed subjects. This suggested to us that a dioxins-Ahr-IL-6/IL-22 axis should be taken into account when evaluating any consequent effects of dioxins. It is presumed there is an Ahr-inflammatory cytokine regulatory feedback loop wherein Ahr activation is able to impact on production of IL-1β, TNFα, IL-6, and IL-22 even while these inflammatory cytokines are potent factors modulating Ahr signaling itself. Further investigation is needed to clarify if increased Ahr expression induced by dioxins is a cause of inflammatory cytokine elevation/reduction or a result of it.

As dioxins (TCDD and many other various forms in this class) are retained in hosts for a long time, there is a great chance of impact on human health, to some extent, for a long period of time following single/multiple exposures to this toxicant (Warner et al. 2015; Wesselink et al. 2014; Mitoma et al. 2015; Landgren et al. 2015). Here, it was documented that RA was among the various pathologies whose incidence was elevated in the evaluated Vietnamese subjects. It has been reported that dioxins may exacerbate Ahr-mediated RA pathogenesis (Kobayashi et al. 2008; Esser et al. 2009; Nguyen et al. 2015; Perricone et al. 2016). As such, it is thus understand-able then that Ahr expression was found to be slightly increased in dioxin-exposed people with RA compared to in those without RA. Such outcomes likely reflect a potential key role for Ahr in RA development in dioxin-exposed people that seem related to an earlier finding showing that Ahr deficiency suppressed development of CIA (model of RA) in mice (Nakahama et al. 2011).

Lastly, a recent study reported there were differential expression patterns between pro-inflammatory Th17 and immunosuppressive Treg cells for Ahr expression - and that the patterns were dependent on host immune status (Cheng et al. 2017). Specifically, Ahr is more abundantly expressed on Treg than on Th17 cells in healthy people. However, in RA patients, Ahr is more highly expressed on Th17 cells. This could suggest that RA patients would be increasingly susceptible to effects of dioxins, and in a vicious cycle, this would then lead to increases in Th17 cell differentiation and RA itself. Therefore, Th17 cells and its secretory cytokines such as IL-17 should be taken into account for treatment of inflammatory diseases including RA in dioxin-exposed people.

Lastly, it has been recently noted that several compounds isolated from a Vietnamese medicinal plant (Eurycoma longifolia) could impart potent anti-inflammatory effects, including inhibition of IL-6 production (Ngoc et al. 2016; Nguyen et al. 2016). It is known that some plant-derived compounds have antagonistic effects against dioxins-mediated CYP1A1 induction (El Gendy et al. 2012, El Gendy and El-Kadi 2013). In addition, several plant compounds have been also found to reduce symptoms of CIA in mice by leading to decreases in IL-17 production (Cho et al. 2009; Tu et al. 2012; Tabarkiewicz et al. 2015). Whether E. longifolia-derived compounds are Ahr antagonists [capable of inhibiting Th17 cells/IL-17 directly or indirectly by blocking inducers such as IL-6] that could consequently become potential therapeutics for RA in the dioxins-exposed people still living near Da Nang airbase is currently under investigation.

Note to readers

Unfortunately, a limitation of this study is a lack of information about the smoking status or use of specific medications of the subjects. This may have resulted in a weakened estimation of the potential relation between dioxin exposure, gene expression of Ahr and inflammatory cytokines, and disease incidence in dioxin-exposed people. Despite this weakness, this study is one of the first to have examined the possible effects of dioxins on resident Vietnamese residing near still-highly dioxin-exposed areas. Other published papers have reported on effects of dioxins in patients lacking several confounding factors, including smoking (see Tai et al. 2013), while others did not (see Fukushima et al. 2016). In future studies, efforts will be made to control for such confounding factors as smoking or medication use in order to obtain a much clearer linkage between dioxin exposure and measured outcomes among the dioxin-exposed Vietnamese populations.

Conclusions

This study showed there were significantly higher levels of Ahr expression, increased gene expression levels of IL-1β, TNFα and IL-6 and decreased levels of IL-22 in samples from dioxins-exposed people than in non-exposed controls. Building on the findings, it is clear that larger-scale studies are required to consider if these changes in Ahr expression might be a useful indicator for local contamination with dioxins. Furthermore, how an increased Ahr expression alters inflammatory cytokine levels and whether it consequently affects the incidence of diseases, including RA, in people exposed to dioxins requires more extensive investigation as well.

Disclosure statement

The authors declare no conflicts of interest. The authors alone are responsible for the content of this manuscript.
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