NPC1 repression contributes to lipid accumulation in human macrophages exposed to environmental aryl hydrocarbons

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Received 26 May 2008; revised 8 December 2008; accepted 29 December 2008; online publish-ahead-of-print 8 January 2009

Time for primary review: 22 days

Aims Aryl hydrocarbons (AHs), such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and benzo(a)pyrene (BP), are environmental contaminants promoting the development of atherosclerosis-related cardiovascular diseases. In order to identify molecular mechanisms involved in these effects, we have analysed AH-mediated regulation of the lipid trafficking Niemann–Pick type C1 protein (NPC1) and its contribution to AH-induced macrophage lipid accumulation.

Methods and results Exposure of primary human macrophages to TCDD and BP decreased NPC1 mRNA expression in a time-dependent manner. NPC1 protein expression and NPC1-related acid sphingomyelinase activity were reduced in parallel. NPC1 was also similarly down-regulated in mice exposed to BP. Moreover, TCDD and BP were demonstrated to trigger lipid accumulation in human macrophages, as assessed by Oil Red O and Nile Red staining and cholesterol determination. Such lipid loading occurred at least partly in endosomal/lysosomal compartments as demonstrated by immunolabelling of lipid vesicles by the lysosome-associated membrane protein 1. These cellular phenotypic effects were found to be similar to those triggered by knock-down of NPC1 expression using siRNAs and were counteracted by NPC1 overexpression, thus supporting the contribution of NPC1 to AH-induced macrophage lipid accumulation in macrophages. Finally, both NPC1 down-expression and lipid accumulation in response to TCDD were found to be abolished through knock-down of the AH receptor (AHR), a ligand-activated transcription factor mediating many effects of AHs.

Conclusion Our data have shown that contaminants such as TCDD and BP repress NPC1 expression in macrophages in an AHR-dependent manner, which likely contributes to macrophage lipid accumulation caused by these environmental chemicals. Thus, NPC1 appears to be a new molecular target regulated by environmental AHs and putatively involved in their deleterious cardiovascular effects.

KEYWORDS
Aryl hydrocarbon receptor; Lipid accumulation; Macrophage; Niemann–Pick type C1; TCDD

1. Introduction

Aryl hydrocarbons (AHs) such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and polycyclic AHs (PAHs), which constitute major environmental contaminants, are especially found in cigarette smoke, automobile diesel exhausts, urban atmospheric particles and the human diet.¹,² These contaminants are unfortunately well known to exert various toxic effects on human health. They are notably thought to favour the development of atherosclerosis-related cardiovascular diseases.³,⁴

Benzo(a)pyrene (BP), a prototypical PAH, has thus been shown to accelerate atherosclerotic plaque development in apolipoprotein-E knock-out mice, via promoting a local inflammatory response,⁵ and inducing DNA damage in human blood vessels.⁶ Moreover, TCDD has been recently demonstrated to promote the transformation of macrophages to foam cells,⁷ which are well recognized as key actors, in the atherosclerotic process.⁸ In addition, BP and TCDD induce expression of pro-inflammatory chemokines such as IL-8,⁹ CCL-1,¹⁰ and MCP-1,¹¹ well known to contribute to atherosclerosis; this occurs through activation of the cytosolic AH receptor (AHR), therefore, triggering its translocation into the nucleus and its subsequent association with its partner coactivator, AHR nuclear translocator (ARNT). The AHR/ARNT heterodimer then binds to its recognition DNA sequence, termed the xenobiotic responsive element (XRE), leading to transcriptional regulation of responsive genes.¹²

The exact nature of these responsive genes, targeted by TCDD and/or PAHs and presumed to contribute to their
cardiovascular effects, remains however incompletely characterized. To address this issue, we have recently analyzed the transcriptome of BP-treated human macrophages using macroarrays. Data from these experiments suggest that expression of Niemann-Pick type C1 protein (NPC1) may be down-regulated by AHR agonists in blood monocytes-derived macrophages, which represent a well-established model for investigating atherosclerosis-related processes. Interestingly, NPC1 is a transmembrane protein containing a sterol-sensitive domain that participates in cholesterol trafficking from the late endosome/lysosome to the plasma membrane, and is therefore thought to protect from cholesterol intracellular accumulation. Niemann-Pick type C disease, an autosomal recessive disorder principally caused by NPC1 mutations, is consequently characterized by accumulation of unesterified cholesterol and other lipids in late endosomes and lysosomes. This underlines the relationships between NPC1, cholesterol homeostasis, and lipid accumulation and therefore the interest to fully characterize a putative regulation of NPC1 by environmental atherogenic compounds such as PAs and TCDD.

In the present study, we have fully confirmed that exposure to AHR agonists such as TCDD and BP decreases expression of NPC1 in an AHR-dependent manner. Such a repression was moreover shown to be most likely involved in lipid accumulation caused by TCDD in human macrophages, suggesting that down-regulation of NPC1 levels by TCDD in human macrophages, with the concomitant repression of NPC1 by environmental atherogenic compounds such as PAs and TCDD.

2. Materials and methods

2.1 Chemicals

BP, benzo(e)pyrene (BeP), progesterone, Oil Red O (ORO) and actinomycin D were provided by Sigma-Aldrich (St. Louis, MO, USA). TCDD was obtained from Cambridge Isotope Laboratories (Cambridge, MA, USA).

2.2 Isolation and culture of human macrophages and animal treatment

Monocytes were purified from peripheral blood mononuclear cells obtained from blood buffy coats (provided by Etablissement Français du Sang, Rennes, France), as previously described. Primary human macrophages were then obtained by GM-CSF-mediated differentiation of monocytes for 6 days; they were cultured in RPMI 1640 medium supplemented with 10% decomplemented foetal calf serum, except 24 h before and during treatment by AHS, where medium was replaced by a serum-free medium. The experiments were done in accordance with the World Medical Association Declaration of Helsinki.

Animal treatment was realized as previously described. Briefly, adult C57Bl/6 mice were treated once a day by intranasal administration of 500 μg BP or of the solvent vehicle alone and tissues were removed and kept at −80°C. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health guidelines (NIH publication No. 85-23, revised 1996).

2.3 Oil Red O staining

Macrophages were incubated with filtered ORO solution (0.6 g/L ORO in 60% isopropanol) for 10 min and with 1 mL of Mayer’s hemalum (Merck, Darmstadt, Germany) for 1 min. Cells were then washed twice in phosphate-buffered saline (PBS), mounted with microscopy Aquatex (Merck) and observed on a ZEISS axiolab microscope. Cells containing at least ten visible lipid vesicles were counted as ORO-positive.

2.4 Nile Red staining

Cells were scrapped and centrifuged at 4500 rpm at 4°C for 5 min. Supernatants were discarded and pellets were incubated with Nile Red solution (Invitrogen, Cergy-Pontoise, France) at the final concentration of 1 mg/L for 10 min. After washing, cellular fluorescence was measured at 584 ± 20 nm by flow cytometry (FACScalibur, Becton Dickinson, Le Pont de Claix, France) and expressed as mean of fluorescence intensity (MFI).

2.5 Cholesterol dosage

Cellular cholesterol content was evaluated using the Cholesteryl/cholesterol ester quantitation kit from Calbiochem (La Jolla, CA, USA), based on a fluorometric cholesterol oxidase method, according to the instructions provided by the manufacturer.

2.6 Transfection of siRNAs and plasmids

siRNA oligonucleotide sequences directed against human ARNT (iARNT) (reference L-007207), NPC1 (iNPC1) (reference L-008047) and a not targeted sequence (iNT), used as control, were from Dharmacon (Lafayette, CO, USA), whereas siRNA sequence directed against human AHR (iAHR) was provided by Genseys (Sigma-Aldrich) and was as follows: 5’-AAGUCGUGUCUAGUGCGCTT-3’; pCMV6-XL4 NPC1 (pCMV-NPC1) corresponded to a human NPC1 expression plasmid (Origene, Rockville, MD, USA) whereas pCDNA3 was used as plasmid control and pmaxGFP, a green fluorescent protein (GFP) expression plasmid, as a control of transfection efficiency. Transfection of pCDNA3 (5 μg), pCMV-NPC1 (5 μg), pmaxGFP plasmid (1.5 μg) or siRNAs (2 μg) into macrophages was performed via Nucleofector technology (Amaxa GmbH, Köln, Germany), according to the manufacturer’s instructions. 24 h after nucleofection, culture medium was changed for additional 48 h; macrophages were then exposed to TCDD for 24 h or 48 h for RNA and protein expression analysis and ORO-staining, respectively.

2.7 RNA isolation and reverse transcription-real time quantitative PCR analysis

Total RNA were isolated from primary macrophage,s using the TRIzol method (Invitrogen) or from mouse jejunum using the FastRNA Pro Green kit (Biogene, Kimbolton, UK). They were then subjected to reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis as previously described. Gene-specific primers were intron-spanning for NPC1 and were as follows: sense: 5’-GGTCCGCTGTGTTACGTTT-3’; antisense: 5’-GGCTTACCCAGTCCAAATA-3’. CYP1A1 and 18S primer sequences were exactly as previously described. Amplification curves of the PCR products were analysed with the ABI Prism SDS software using the comparative cycle threshold method. Relative quantification of the steady-state target mRNA levels was calculated after normalization of the total amount of cDNA tested to an 18S RNA endogenous reference.

2.8 Western blot analysis

Total cellular protein preparation and western blot were performed as previously described, using anti-AHR (Biomol Research Labs, Plymouth, PA, USA), anti-NPC1 (Novus Biologicals, Littleton, CO, USA), anti-ARNT, anti-β-Actin and anti-HSC70 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) antibodies. After incubation with appropriate horseradish peroxidase-conjugated secondary antibodies for 2 h, immunolabelled bands were visualized by autoradiography using chemiluminescence.
2.9 Acid sphingomyelinase assay
Acid sphingomyelinase (ASM) activity was performed using a kinetic assay, exactly as previously described. Briefly, this method measures the release of radioactive phosphocholine, the hydrolysis product of the ASM substrate choline-methyl-[14C]SM, through scintillation counting. ASM activity was expressed as cpm/μg protein.

2.10 Fluorescence microscopy
After treatment, cells were fixed in 4% paraformaldehyde (Sigma-Aldrich) for 15 min and then washed in PBS. For NPC1, lysosome-associated membrane protein 1 (LAMP-1) and adipophilin immunolocalization, cells were pre-incubated with PBS-bovine serum albumin 4%, saponine 0.4% for 1 h before incubation overnight with anti-NPC1, anti-LAMP-1 (Santa Cruz Biotechnology) or anti-adipophilin (Progen Biotechnik, Heidelberg, Germany) antibodies or with isotype-control. Cells were then washed in PBS and re-incubated with rhodamine-(TRITC)-labelled donkey anti-rabbit antibody for NPC1 labelling or with FITC-labelled goat anti-mouse antibody for LAMP-1 and adipophilin labelling (Jackson ImmunoResearch Laboratories, Suffolk, UK). Lipid and nuclei were stained by ORO and DAPI, respectively. Fluorescent images of DAPI, GFP, NPC1, LAMP-1, and ORO staining were analysed using a DMRXA Leica microscope and a COHU high-performance CCD camera using a metavue software.

2.11 Statistical analysis
Data were analysed using the Student’s t test or with ANOVA followed by the Dunnett’s t test.

3. Results
3.1 Down-regulation of NPC1 expression in response to TCDD exposure
NPC1 expression was first investigated at mRNA levels in primary human macrophages exposed to various TCDD concentrations (from 0.1 to 10 nM) for 24 h (Figure 1A); such

![Figure 1](image-url)
TCDD concentrations were found not to affect cell viability as demonstrated by light microscopic examination and measurement of cellular exclusion of trypan blue (data not shown). TCDD was shown to moderately decrease NPC1 mRNA expression: a significant down-expression of NPC1 mRNA levels was thus detected after treatment by 1 or 10 nM TCDD whereas, in contrast, the dose of 0.1 nM was not effective (Figure 1A). Kinetic analysis of NPC1 mRNA expression further demonstrated that NPC1 mRNA levels started to significantly decrease after a 16 h TCDD treatment; this decrease was maximal after a 24–36 h exposure to TCDD (Figure 1B). Similarly to TCDD, BP reduced NPC1 mRNA amounts in primary human macrophages whereas, in contrast, BeP, a PAH known to exert no or only weak toxic effects, was not effective (Figure 1C). In vivo administration of BP to mice for 24 and 72 h also resulted in a significantly decreased expression of NPC1 mRNA in PAH-responsive tissue such as intestine (Figure 1D).

We next performed measurements of NPC1 mRNA half-life using actinomycin D to block NPC1 transcription, in the absence or presence of TCDD. As shown in Figure 1E, NPC1 mRNA half-lives in TCDD-treated macrophages and untreated counterparts were similar (10 h 35 min vs. 10 h 45 min, respectively), thus making unlikely that TCDD may act on NPC1 mRNA expression through altering NPC1 mRNA stability. Down-regulation of NPC1 mRNA level was associated with a reduction of NPC1 protein content in TCDD- and BP-treated macrophages, as assessed by western blot analysis (Figure 2A). Densitometry analysis of immunolabelled bands thus indicated that NPC1, detected as an heterogenous 170–220 kDa glycosylated protein, was reduced by 29.9 ± 5.6% and by 29.6 ± 8.9% (n = 4) in TCDD- and BP-treated cells, respectively, when compared to untreated counterparts. Such a decrease of NPC1 expression was most likely not linked to an increased NPC1 degradation since treatment by proteasome inhibitors failed to prevent TCDD-induced protein down-expression (data not shown).

We next measured the activity of ASM, a lysosomal enzyme responsible for sphingomyelin hydrolysis, in TCDD-treated cells, since ASM activity has been shown to be reduced in NPC patients.21 ASM activity was found to be moderately, but significantly, reduced in TCDD-exposed cells when compared to that found in untreated macrophages (Figure 2B), suggesting that NPC1 down-regulation in response to TCDD has functional consequences, i.e. cholesterol-related down-modulation of ASM activity. Progesterone was used in parallel as a positive control mimicking NPC1-related disease22 through increasing accumulation of cholesterol in lysosomes and consequently reducing ASM activity (Figure 2B), as already demonstrated in fibroblasts.23

3.2 Implication of NPC1 down-regulation in lipid accumulation in TCDD- and BP-exposed macrophages

TCDD has been recently shown to trigger lipid accumulation in macrophagic U937 cells.7 We first investigated whether treatment by this environmental contaminant or by PAHs can also induce lipid accumulation in human primary macrophages. ORO staining indicated that TCDD and BP increased intracellular lipid contents, as detected by light and fluorescence microscopy (Figure 3A, lanes 1 and 2), and thus increased significantly the number of ORO-positive macrophagic cells (Figure 3C); we obtained similar results in macrophages exposed to progesterone, used here as positive controls (Figure 3A–C).22 To investigate intracellular lipid distribution in Ah-exposed macrophages, we next analysed expression of LAMP-1, a late endosomal/lysosomal marker, and of adipophilin, a lipid droplet-associated protein.24 Our results showed that LAMP-1 colocalized with at least some ORO-stained lipid compartments in TCDD-, BP-, and progesterone-treated cells (Figure 3A, lanes 2 and 3). In addition, the presence of adipophilin surrounding some ORO-stained lipids was observed (Figure 3B, lanes 2 and 3). Moreover, adipophilin expression seems increased in macrophages exposed to TCDD, BP, or progesterone when compared to untreated or BeP-treated counterparts (Figure 3B, lane 1); such an up-regulation of adipophilin has been commonly reported in lipid-accumulating cells.25

TCDD- and BP-increased lipid accumulation in macrophages was confirmed by the Nile Red staining method (Figure 3D). TCDD and BP treatment were finally demonstrated to significantly increase cellular content of total and unesterified cholesterol, without altering levels of esterified cholesterol (Figure 3E). In contrast, BeP failed to induce lipid accumulation (Figures 3A, C, and D) or to up-regulate cholesterol content in human macrophages (Figure 3E). Altogether, our data suggested that TCDD- and BP-treated macrophages accumulated unesterified cholesterol and lipids similarly to progesterone-treated macrophages (Figure 3A, C, and D).

To investigate the role potentially played by NPC1 down-regulation in lipid accumulation in TCDD-treated
macrophages, we first realized a knock-down of NPC1 expression by using siRNAs. The down-regulation of NPC1 mRNA levels in macrophages transfected by siRNAs against NPC1 (iNPC1) was about 33.5 ± 4.0% (n = 5) in comparison to macrophages transfected by not targeting siRNAs (INT), used as controls (Figure 4A), and was comparable to the decrease of NPC1 mRNA levels observed in TCDD-treated macrophages (Figure 1C). Efficiency of siRNAs targeting NPC1 was moreover validated by western blot analysis (Figure 4A, inset). As shown in Figure 4B, NPC1 knock-down-induced lipid accumulation, visualized by ORO method, in macrophages; interestingly, this increase in the number of lipid-positive cells was similar to that occurring in response to TCDD exposure in INT-transfected macrophages (Figure 4B). TCDD, however, failed to affect ORO staining in iNPC1-transfected macrophages (Figure 4B).

To more precisely investigate the potential role of NPC1 in TCDD-induced formation of macrophages foamy cells, we next transfected human primary macrophages with a human NPC1 expression vector (pCMV-NPC1) in the presence of a pmaxGFP expression vector. Because transfection efficiency is unfortunately low in primary human macrophages, we realized two controls. First, we showed by western blot that transfection of the human NPC1 expression vector pCMV-NPC1 increased total cellular level of NPC1 in macrophages (Figure 5A). Secondly, we showed by immunofluorescence staining that pCMV-NPC1/GFP-positive cells, exposed or not to TCDD, expressed more NPC1 than pcDNA3/GFP-positive cells.

![Figure 3](image-url) Up-regulation of lipid content by AHR agonists. Macrophages were either untreated (UNT) or treated with several AHRs (1 μM BP, 10 nM TCDD, and 2 μM BeP) and with 35 μM progesterone (Prog) for 48 h. Lipid accumulation was observed through light (A; lane 1) and (C) and fluorescence (A and B, lanes 2 and 3) microscopy after ORO staining. The distribution of cellular lipid was determined by staining cells with ORO (red fluorescence) and anti-LAMP-1 antibody (green fluorescence) (A, lanes 2 and 3 which corresponds to an enlargement of the white square of lane 2 pictures) or by staining cells with ORO (red fluorescence) (B, lanes 2 and 3) and anti-adipophilin antibody (green fluorescence) (B, lanes 1 and 3) and then by performing fluorescence microscopy; pictures acquired and processed using identical conditions are representative of three independent experiments. (B) Colocalization of ORO and adipophilin was indicated by arrows and free-adipophilin area containing ORO-stained lipids were indicated by asterisks. (C) Data expressed as the percentage of ORO-positive cells are the means ± SEM of six independent experiments. (D) Neutral lipid accumulation was evaluated by measuring Nile Red fluorescence by flow cytometry; data are expressed as a percentage of the levels found in untreated cells, and are the means ± SEM of seven independent experiments; (D, Inset) flow cytometric graphs for TCDD- and progesterone-treated cells (white picks) and corresponding control untreated cells (grey picks) are provided. (E) Total unesterified and esterified cellular cholesterol were determined using a colorimetric method; data expressed relative to the total protein concentration are the means ± SEM of three independent experiments. *P < 0.05 when compared with untreated cells.
3.3 Involvement of AHR in down-modulation of NPC1 expression and lipid accumulation occurring in TCDD-treated macrophages

In order to investigate the role played by AHR in both NPC1 down-regulation and lipid accumulation caused by TCDD, we used primary macrophages where expression of AHR or of its nuclear partner ARNT have been markedly abolished using appropriate siRNAs, as fully validated by western blots using anti-AHR and anti-ARNT antibodies, respectively (Figures 6A and C). Basal mRNA expression of NPC1 was not modified by knock-down of AHR or ARNT (Figures 6B and D). However, we found that the decrease of NPC1 mRNA expression occurring in response to a 24 h TCDD treatment was abolished in iAHR- and iARNT-transfected macrophages (Figures 6B and D), suggesting that AHR and ARNT are most likely required for TCDD-mediated down-regulation of NPC1 expression. In a similar way, TCDD failed to induce lipid accumulation in iAHR-transfected macrophages (Figure 6E). The number of ORO-positive cells was moreover similar in the absence of TCDD in both iAHR-macrophages and control iNT-cells (Figure 6E), indicating that AHR knock-down had no effect by itself on basal lipid accumulation.

4. Discussion

Our results demonstrate that NPC1, an intracellular cholesterol traffic protein, is a new molecular target for the major environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). We have shown that NPC1 overexpression prevents TCDD-induced lipid accumulation in human macrophages.
environmental contaminants TCDD and BP. Indeed, treatment of human macrophages by these AHs was found to decrease expression of NPC1 at both mRNA and protein levels in a moderate, but significant, manner. Moreover, ASM activity, which is down-regulated in cells from NPC-deficient patients, was also found to be reduced in TCDD-treated macrophages. This point brings indirect functional evidence for a repression of NPC1 expression in TCDD-exposed macrophages.

NPC1 down-regulation by TCDD does not involve a decrease of NPC1 mRNA half-life, suggesting that the environmental contaminant may act on NPC1 mRNA expression at a transcriptional level rather than at a post-transcriptional level; in agreement with this conclusion, our preliminary data indicate that TCDD treatment reduces NPC1 promoter activity (data not shown). Transcriptional regulations are usually the rule for genes targeted by TCDD and PAHs via AHR activation. In this context, it is noteworthy that AHR is involved in NPC1 down-regulation by TCDD. Indeed, both TCDD and BP, which are well considered as potent AHR agonists, reduced NPC1 expression, whereas, by contrast, BeP, known to not or only poorly interact with AHR, failed to change NPC1 mRNA levels. Moreover, knock-down of AHR or of its partner ARNT using siRNAs, fully

Figure 5  Inhibition of TCDD-induced lipid accumulation by NPC1 overexpression. Macrophages were co-transfected with pmaxGFP and pCDNA3 or pCMV-NPC1 plasmids. (A) NPC1 protein expression was analysed by western blot; data shown are representative of three independent experiments. (B–D) Transfected macrophages were either untreated (UNT) or treated by 10 nM TCDD for 24 h, NPC1 expression (B) and lipid accumulation (C) were then analysed using anti-NPC1 antibody and ORO staining, respectively, and were visualized in GFP-positive cells by fluorescence microscopy; pictures acquired and processed using identical conditions are representative of three independent experiments. (D) Means of fluorescence intensity (MFI) of ORO-related staining in GFP-positive cells were expressed as percentages of the levels found in untreated cells, and are the means ± SEM of values from at least 15 GFP-positive independent cells for each condition, from three independent experiments.*P < 0.05; NS, not significant.
prevented NPC1 mRNA down-regulation in TCDD-treated macrophages. The exact mechanism by which AHR reduces NPC1 expression in macrophages remains however to be determined. Interestingly, in silico analysis of human NPC1 promoter (NM_000271) reveals the presence of several XRE consensus (5'-GCGTG-3') sites,26 commonly found in the promoter sequence of genes targeted by AHR agonists and known to bind the AHR-ARNT complex; such a binding however usually primarily results in induction of TCDD-responsive genes, and not in a reduction of gene expression as observed for NPC1. This suggests that NPC1 is regulated by AHR through a non-classical pathway or by an indirect mechanism, i.e. TCDD may first up-regulate a yet undetermined factor in a classical AHR-dependent manner, which may contributes secondary to down-regulation of NPC1 expression. The fact that down-expression of NPC1 by TCDD is a relatively late event, i.e. it occurred after a 16–24 h treatment by TCDD, may support this hypothesis.

Exposure to TCDD and PAH-containing diesel particles has been previously shown to trigger lipid accumulation in transformed macrophagic U937 cells.7,27 The present study extended this observation to normal macrophages. Interestingly, a loss of NPC1 function has been shown to decrease cholesterol efflux via ABCA1 and ABCG1 transporters, leading to lipid accumulation in human macrophages.13 A direct contribution of NPC1 down-regulation to AH-induced lipid accumulation in primary human macrophages has therefore to be considered, even if the down-regulation of NPC1 expression in response to AHs is rather moderate. This conclusion is notably supported by the following points: (i) exposure to TCDD or BP increased lipid accumulation in at least some late endosome/lysosome compartments and enhanced unesterified cholesterol content, which constitute recognized hall-marks of NPC1-deficient cells,14 (ii) knock-down of NPC1 expression using siRNAs, which quantitatively resulted in a down-regulation of NPC1 mRNA close to that occurring in response to TCDD, resulted in a foamy phenotype of macrophages similar to that observed in response to AHs, (iii) overexpression of NPC1 prevented TCDD-triggered accumulation of lipids in macrophages, (iv) AHR, required for NPC1 regulation by TCDD,
was also involved in the accumulation of lipids in TCDD-treated macrophages as shown by decreased ORO staining of iAHR-transfected cells and (v) among PAHs, BP, which reduced NPC1 in a way similar to TCDD, also triggered lipid accumulation in macrophages, whereas BeP that failed to act on NPC1, also failed to induce lipid amount. Besides NPC1 repression, other molecular pathways can contribute to lipid accumulation in AH-macrophages. Down-regulation of cholesterol efflux via decreased expression of ABCA1 and ABCG1 transporters or altered function of Niemann–Pick disease type C2 protein (NPC2) are however unlikely to play a role in TCDD-induced lipid accumulation in macrophages since TCDD failed to alter mRNA expression of ABCA1, ABCG1, or NPC2 (data not shown). By contrast, AH-treated macrophages were found to exhibit some lipid vesicles stained by adophilin, which may evoke the formation of lipid droplets in response to AhR agonists. This formation of adophilin-related lipid vesicles may be indirectly related to NPC1 repression and to its functional consequences such as enhanced content of cellular lipid, which is known to up-regulate adophilin expression and subsequently to trigger the formation of lipid droplets. Further studies would be required to investigate this hypothesis.

It is noteworthy that alteration of cellular lipid trafficking due to NPC1 repression can contribute to atherosclerosis, as recently demonstrated in mice. This suggests that NPC1 down-regulation in response to AHS and the subsequent accumulation of lipids into macrophages can likely participate to the pro-atherogenic effects of these environmental contaminants. The fact that the doses of TCDD or BP acting on NPC1, i.e. 1 nM and 1 μM, respectively, are close to those found in some foods, in cigarette smoke or in industrial wastes, fully supports this conclusion, suggesting that humans are likely exposed to TCDD or PAH concentrations affecting NPC1 expression and, by this way, putatively triggering lipid accumulation. In addition, it should be kept in mind that other phenotypic effects of AHSs, including up-regulation of proinflammatory cytokines, can favor the development of cardiovascular diseases. Taken together, these data indicate that several distinct mechanisms may contribute to cardiovascular toxicity of these environmental contaminants.

In summary, NPC1 was identified as a molecular target repressed by some environmental AhR ligands such as TCDD and BP, which likely contributes to lipid accumulation caused by these contaminants in macrophages. By this way, NPC1 may therefore be involved in deleterious atherosclerosis-related cardiovascular effects of environmental AHSs.

Acknowledgements
We wish to thank L. Vernhet, M.T. Dimanche-Boitrel, and D. Gilot for helpful comments, and C. Morzadec and M. Le Vée for expert technical assistance.

Conflict of interest: none declared.

Funding
This work was supported by the Ligue Nationale contre la Cancer. N.P. and A.R. are recipients of fellowships from the Région Bretagne.

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