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The Aryl Hydrocarbon Receptor Has an Important Role in the Regulation of Hematopoiesis: Implications for Benzene-induced Hematopoietic Toxicity

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Abstract

The aryl hydrocarbon receptor (AhR) belongs to the basic-helix-loop helix (bHLH) Per-Arnt-Sim (PAS) family of transcription factors. Many of these proteins are involved in regulating responses to signals in the tissue environment such as hypoxia, oxidation-reduction status, and circadian rhythms. Although the AhR is well-studied as a mediator of the toxicity of certain xenobiotics, the normal physiological function remains unknown. However, accumulating data support a hypothesis that the AhR has an important function in the regulation of hematopoietic stem cells (HSCs). Persistent AhR activation by dioxin, a potent xenobiotic AhR agonist, results in altered numbers and function of HSCs in mouse bone marrow. Analysis of HSCs from AhR null-allele mice also indicates that lack of AhR expression results in altered characteristics and function of these cells. HSCs from these animals are hyperproliferative and have altered cell cycle. In addition, aging AhR-KO mice show characteristics consistent with premature bone marrow senescence and are prone to hematopoietic disease. Finally, some data suggest that the expression of the *Ahr* gene is regulated under conditions that control HSC proliferation. The presence of a normal functioning AhR may provide an important advantage to organisms by regulating the balance between quiescence and proliferation and preventing the premature exhaustion of HSCs and sensitivity to genetic alterations. This function assists in the preservation of HSC function and long-term multi-lineage generation over the lifespan of the organism. This also implicates a role for the AhR in the aging process. Furthermore, these functions may affect the sensitivity of HSCs to certain xenobiotics, including benzene. Defining the molecular mechanisms by which these events occur may lead to the identification of previously undefined roles of this transcription factor in human diseases, particularly those caused or affected by xenobiotics.

Keywords

aryl hydrocarbon receptor; benzene; hematopoietic stem cells

1. Introduction

The hematotoxic effects of benzene in both exposed humans and experimental animals are well known [1,2]. Most of the investigations on the mechanisms by which benzene may produce

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these effects have focused on the metabolism of benzene and the ability of these metabolites to bind to DNA and protein [3,4], and/or initiate redox cycling and produce reactive oxygen species (ROS) [5]. More recent data support the hypothesis that benzene-induced hematotoxicity results from the generation of ROS by benzene metabolites, particularly those conjugated with glutathione [6], and subsequent oxidative stress.

Several years ago it was noted that *Ahr* null-allele (KO) mice failed to show hematotoxicity following exposure to benzene [7]. This same group also reported that benzene failed to induce hematotoxic effects in lethally-irradiated wild-type mice that had been transplanted with bone marrow cells from AhR-KO animals [8]. These data are especially relevant given the ability of this ligand-activated transcription factor to regulate the expression of a number of enzymes responsible for the bioactivation of certain xenobiotics that can generate electrophiles and redox cycling [9]. One of the cytochrome P450 (CYP) enzymes, CYP2E1, has been shown to metabolize benzene to a number of reactive metabolites including phenol, catechol, and hydroquinone [10]. Since both the AhR and CYP2E1 are present in primitive hematopoietic progenitor cells [11–14], it has been postulated [8] that the AhR plays a significant role in the susceptibility to benzene through the ability to regulate, directly or indirectly, CYP2E1 expression in these cells. However, there does not appear to be a significant difference in CYP2E1 expression between wild type and AhR-KO mice [7]. Variations in the tissue levels of two AhR-regulated enzymes, NAD(P)H:quinone oxidoreductase (NQO1) and glutathione-S-transferase (GST) [15,16] that are involved in the detoxification of benzene metabolites, have also been shown to modify benzene toxicity [17–19]. It is possible that the altered expression of these enzymes by the AhR and/or activation of the AhR by benzene metabolites could contribute to differential susceptibility to benzene. However, other studies indicate that hydroquinone or benzoquinone do not activate the AhR, and that the presence or absence of the AhR in mouse hepatoma cells did not affect ROS production induced by these benzene metabolites [20]. However, this study has not been performed in bone marrow cells. Thus, although it appears the AhR has an important function in regulating susceptibility to benzene-induced hematotoxicity through its presence and activity in bone marrow cells, the specific role is not known.

The AhR has been known primarily for its role in the regulation of several drug and xenobiotic metabolizing enzymes, as well as the mediation of the toxicity of certain xenobiotics, including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). In addition, accumulating evidence strongly suggests that the AhR has subtle, but critical functions in the development and maintenance of a number of tissues including vasculature, liver and heart [21–25]. There is also much evidence to indicate that the AhR has a role in regulating immune responses, particularly those involved in anti-viral immunity and regulatory T-cells that modulate autoimmune and allergic disease [26–30]. The AhR seems to be required for optimal resistance of mice to *Listeria* infection [31]. Here we summarize recent data supporting a novel hypothesis that the AhR has an important function in the regulation of hematopoietic stem cells (HSCs). Further defining this role will have important implications for understanding not only mechanisms underlying benzene-induced toxicity, but those processes that regulate and preserve HSC function throughout the lifetime of an organism.

2. Exposure to xenobiotic AhR ligands affects hematopoietic processes and HSC function

There have been several studies reporting an association between increased incidence of leukemia and lymphoma in human populations accidentally exposed to TCDD and other related halogenated polycyclic aromatic hydrocarbons [32–36]. Leukemia is also increased in human offspring exposed during pregnancy to tobacco smoke, which contains numerous AhR ligands [37–40]. However, causality between exposure to these particular chemicals and the

development of any hematopoietic disease mediated by the AhR in humans has not been proven. Furthermore, the cellular and molecular mechanisms remain unknown; it is unclear whether these ligands are acting directly on human bone marrow cells.

On the other hand, the cumulative animal data are consistent with xenobiotic AhR ligands affecting hematopoietic processes by their direct action on stem and/or progenitor populations (summarized in Table 1). In this review, only TCDD is considered since other AhR ligands, such as benzo[a]pyrene and other PAHs, may elicit additional effects not solely related to AhR activation because of the production of their biologically active metabolites. In addition to the actions of TCDD on specific biochemical and cellular endpoints in bone marrow, as indicated in Table 1, it is most significant that TCDD exposure to mice affects the function of HSCs as determined by their ability to engraft into lethally irradiated animals [13,48]. This effect alone and the additional finding that this is dependent on the presence of the AhR in hematopoietic cells [48], strongly suggest that TCDD is directly targeting the AhR within these stem cell populations to alter their function. The finding that TCDD alters gene expression in these cells [48] is further evidence of this. However, the successful repopulation of HSCs into bone marrow is dependent on a number of events including migration from the circulation through endothelial cells of the bone marrow sinusoids, as well as retention and engraftment into the bone marrow niche. These cells must then be able to respond to signals, soluble and cellular, present in the marrow environment to differentiate and proliferate into more mature populations. Any one or several of these events could be affected by TCDD through the AhR. The additional observation that TCDD exposure disrupts the cycling of HSCs to result in a decreased number of cells in quiescence [47] is relevant to this for several reasons. First, under normal resting conditions the regulation of HSC division is tightly regulated with the majority (70–85%) of cells being in the quiescent state (G_0 phase of cell cycle). This is critical for the maintenance of hematopoietic potential throughout the lifespan of the organism. Second, several studies noted a relationship between cycling status of HSCs and engraftability, with quiescent cells having greater ability for engraftment [49,50]. As such, it may not be surprising that HSCs from TCDD treated animals are less able to engraft into bone marrow.

Since most, if not all, of the toxicity caused by TCDD appears to be mediated through its ability to bind to the AhR and alter gene expression, it is reasonable to suspect that the persistent activation of the AhR in HSCs results in the modified expression of genes that are involved in cell cycle regulation and/or the expression of cell adhesion molecules that are important in HSC trafficking and engraftment [51]. Given the large number of genes that are directly or indirectly regulated by the AhR [29,52,53] there are several likely suspects, although the ability of TCDD to affect HSC function may depend on the modulation of a number of several signaling pathways simultaneously. Some of our preliminary data suggest that AhR dysregulation by TCDD may affect the CXCR4/CXCL12 signaling pathway (unpublished observations). This is particularly interesting since this pathway is essential to HSC trafficking and movement in the bone marrow niches and maintenance of a quiescent HSC pool [54]. Genes encoding c-Myc and HES1 are also regulated by the AhR [55,56], and these proteins are known to influence HSC characteristics and function [57,58]. HES1, in particular, has been shown to regulate the balance between cellular quiescence and proliferation [59].

For the purpose of this review, it is important to emphasize that the effects of TCDD on bone marrow may be very different than that produced by benzene. TCDD is metabolized very slowly, and most, if not all, of the toxic effects are elicited through the binding of the parent compound to the AhR and the subsequent modulation of gene transcription. With benzene, however, it seems clear that metabolites are the causative agents. In addition to any possible effects that might be due to the binding of these metabolites to the AhR, reactive benzene metabolites are likely to produce other effects related to their ability to covalently bind to DNA and protein, as well as initiate redox cycling to produce ROS.

3. Regulation of AhR presence and activity in HSCs

Clearly more work is necessary to determine the gene changes elicited by TCDD that are causally associated with alterations in HSC function. However, this task becomes more daunting when one considers that HSCs represent only a very small percentage of bone marrow cells. For example, HSCs are often phenotypically defined as being Lineage⁻, Sca-1⁺, and c-kit⁺ (LSK) as determined by flow cytometry, but LSK cells are really only enriched in true hematopoietic stem cells. As such, there are likely several different populations of progenitor cells within any phenotypically-defined subset. Furthermore, these cells are likely to be at various stages of cycling. If the level and/or activity of the AhR is differentially regulated within these subpopulations or at different stages of the cell cycle, then the susceptibility to AhR ligands may likewise vary.

A number of investigations have shown that AhR levels vary in a cell- and tissue-specific-manner, as well as with stage of development and age. Furthermore, within the same cell type, these levels have been found to change with cell density, state of differentiation, presence or absence of growth factors, and neoplastic transformation [52,60,61]. Several of the mechanisms controlling AhR levels and activity have been well studied (Table 2). Given that these mechanisms have been demonstrated in a number of different cell types, it would be expected that similar events occur in HSCs. The gene encoding the AhR Repressor is expressed in HSCs and this expression is controlled by circadian rhythms [47]. Furthermore, the *Ahr* gene appears to be down-regulated during the proliferative phase of HSCs [13,69,70]. Thus, HSC susceptibility to the actions of some xenobiotic ligands may depend on the marrow environment. Conditions in which HSCs are proliferating may actually protect the cells from toxic AhR ligands or chemicals that are activated to toxic intermediates by enzymes induced by the AhR. In fact, we found that stimulating HSCs to proliferate actually protected them from the toxic effects of TCDD [13]. Likewise, if benzene hematotoxicity is dependent on the presence and activity of the AhR [7,8], the down-regulation of this transcription factor during different phases of the cell cycle and during different stages of differentiation may also render these cells less susceptible to benzene and/or its metabolites.

4. HSC characteristics and age-related hematopoietic patterns are altered in *Ahr* null-allele mice

Although the *Ahr* gene appears to be down-regulated during the proliferation of HSCs, it is not known if this is secondary to changes in other regulatory pathways or is a necessary event in the process of going from quiescence to proliferation. If the down-regulation were an important regulatory event controlling the balance between quiescence and proliferation, one would expect to see altered characteristics of HSCs from AhR-KO mice. We have found that AhR-KO mice have increased numbers of phenotypically-defined LSK and LSK/CD34⁺ progenitor cells in the bone marrow compartment compared with wild-type mice (unpublished observations). Consistent with this, KO mice also have increased numbers of functionally-defined primitive progenitors (HPP-CFC). Perhaps more importantly, LSK cells in KO animals appear to have very high rates of cell division as determined by the incorporation of bromodeoxyuridine (BrdU). Notably, this rate was found to be identical to HSCs from wild-type animals treated with 5-fluoruracil (5-FU) that kills more mature rapidly dividing cells and stimulates HSCs into division. Consistent with this, there is also a significantly greater percentage of KO LSK cells in the G₁/S phases of the cell cycle as compared to those cells from wild-type animals. Furthermore, progenitor cells from KO mice have increased growth rates in culture compared to cells from wild-type animals (unpublished observations), further suggesting that the high proliferation rate of KO HSCs is a property inherent to these cells and not due to changes in other signaling processes present in the bone marrow niche of the KO mice.

Together, these data support the hypothesis that the AhR has a normal role in the regulation of HSCs. More specifically, the data are consistent with the AhR being a negative regulator of the balance between quiescence and proliferation. That is, the AhR may normally serve as a “braking” mechanism to control rates of HSC proliferation. In its absence, this regulation is lost, and HSCs proliferate at a rate that would normally be seen only under conditions of extreme hematopoietic stress. Notably, a previous publication reported that the *Ahr* promoter is silenced by hypermethylation in human acute lymphoblastic leukemia cells [71]. The authors also postulated the AhR protein to be a negative regulator of growth and proliferation, and that the down-regulation of this gene by epigenetic mechanisms may significantly contribute to the development of the leukemia.

Notably, there is much data to suggest a causal relationship between low to moderate cycling rates in HSCs and hematopoietic longevity [72,73]. Furthermore, there is an excellent negative correlation between the mean lifespan of various mouse strains and cycling rates in HSCs [73,74]. Thus, animals with high rates of HSC replication tend to have shorter life spans. Many years ago it was also observed that differences in longevity within mouse strains correlated to some degree with differences in *Ahr* polymorphisms. Animals (e.g. C57BL/6 mice) possessing a high affinity receptor (i.e. high affinity for TCDD) have low HSC cycling rates and a longer lifespan, while strains (e.g. DBA/2 mice) having a low affinity receptor have high cycling rates and shorter lifespans [75,76].

If, as our data indicate, KO HSCs have high rates of proliferation, then these cells may undergo premature senescence as aging occurs. In addition to decreased longevity, some signs of premature bone marrow senescence include altered numbers of HSCs, and a skewing of lineages toward myeloid at the expense of lymphoid populations [73,77–80]. Increased cycling of HSCs also correlates with increased incidence of leukemia, lymphoma, and myelodysplastic syndrome in old age [81,82]. Our initial studies indicate that, unlike young adult mice, 10-month old KO mice have <50% fewer LSK cells in bone marrow compared to age-matched wild-type mice. Furthermore, bone marrow from one-year old KO mice have significantly increased numbers of lineage-restricted myeloid progenitors, while the number of B progenitors are profoundly decreased (unpublished observations). In addition, aging KO mice develop a number of lesions consistent with premature marrow senescence including an accumulation of cells of hematopoietic origin in many tissues. In particular, the splenomegaly in KO mice has been characterized as resulting from myeloid hyperplasia [23]. Many of these tissue lesions are consistent with mice in various stages of lymphoid neoplasms [83]. In preliminary studies in which splenocytes from aged KO mice were examined, we have observed alterations in the mRNA levels of several genes including *gfi-1*, *Sh2d3c*, *gata-1*, *p21*, and *c-myc* (unpublished observations). The altered expression of several of these has been associated with leukemia in mice, and/or predisposition to leukemia [84–86]. Finally, early onset of several neoplasms, including lymphomas and hepatomas, and shortened lifespan has been reported in AhR deficient mice [87]. All of these data are further consistent with a normal role of the AhR in the regulation of HSCs, and in particular as a negative regulator of HSC proliferation.

5. Conclusions

Although all data indicates a role for the AhR in benzene-induced hematotoxicity, the mechanisms by which this occurs are not well understood. The AhR clearly regulates the expression of several enzymes involved in the metabolism of benzene to more biologically active intermediates. However, it also seems clear from the data and information presented here that the AhR has a major role in the regulation of hematopoietic stem cells, specifically in controlling the balance between quiescence and proliferation. As such, there are several possibilities by which the altered presence and/or the activity of the AhR could modulate

responses of hematopoietic stem/progenitor cells to benzene. 1) Altered AhR activity may result in the decreased or increased presence of those enzymes responsible for the activation of benzene to toxic metabolites. 2) The regulation of AhR expression and/or activity within subpopulations of stem/progenitor cells may render specific cell populations more or less susceptible to benzene. This effect might be especially profound under conditions of hematopoietic stress in which HSCs are stimulated to proliferate and AhR may be down-regulated. 3) On the other hand, AhR dysregulation in HSCs could exacerbate the hematotoxic effects of benzene by promoting conditions in which stem/progenitor cells undergo abnormal proliferation and differentiation. Thus, any dysregulation in AhR function may promote conditions that could be additive or synergistic to the effects of benzene. For example, decreased expression of the *Ahr* gene by hypermethylation may result in uncontrolled cell growth and proliferation that may be also be permissive for the leukemogenic effects of benzene. 4) AhR regulation in HSCs, and thus overall HSC function as well as benzene susceptibility, could also be very much age dependent. 5) Although *bona fide* endogenous ligands for the AhR have not been identified, one could also propose a scenario in which benzene exposure could alter the generation and/or activity of these ligands in HSCs to alter HSC function. 6) Finally, reactive metabolites of benzene may alter the expression and/or activity of the AhR thus altering the balance between HSC quiescence and proliferation, further promoting a hematotoxic and or leukemogenic event.

Future research is necessary to define the precise role of the AhR in HSCs especially as this may relate to HSC cycling, regulation of the balance between quiescence and proliferation, and senescence of HSCs. This information will lead to the identification of previously undefined functions of this transcription factor in particular hematopoietic diseases, especially those associated with aging and/or the exposure to xenobiotics such as benzene. Since the AhR is ligand activated, there also may be the possibility of developing preventive and/or therapeutic measures to combat these diseases.

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Abbreviations

AhR	aryl hydrocarbon receptor
AhR-KO	AhR-null allele
HPP-CFC	high proliferative potential colony-forming cells
HSCs	hematopoietic stem cells
LSK HSCs	Lin ⁻ Sca-1 ⁺ c-kit ⁺ HSCs
PAHs	polycyclic aromatic hydrocarbons
ROS	reactive oxygen species
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin

References

1. Snyder R. Overview of the toxicology of benzene. *J Toxicol Environ Health, Part A* 2000;61:339–346. [PubMed: 11086937]
2. Snyder R. Benzene's toxicity: a consolidated short review of human and animal studies by HA Khan. *Human Exp Toxicol* 2007;26:687–696.

3. Lutz WK, Schlatter C. Mechanism of the carcinogenic action of benzene: irreversible binding to rat liver DNA. *Chem Biol Interact* 1977;18:241–245. [PubMed: 890848]
4. Subrahmanyam VV, Ross D, Eastmond DA, Smith MT. Potential role of free radicals in benzene-induced myelotoxicity and leukemia. *Free Rad Biol* 1991;11:495–515.
5. Eastmond DA, Smith MT, Irons RD. An interaction of benzene metabolites reproduces the myelotoxicity observed with benzene exposure. *Toxicol Appl Pharmacol* 1987;91:85–95. [PubMed: 2823417]
6. Brunmark A, Cadenas E. Reductive addition of glutathione to p-benzoquinone, 2-hydroxy-p-benzoquinone, p-benzoquinone epoxides. Effect of the hydroxyl- and glutathionyl substituents on p-benzoquinone autooxidation. *Chem Biol Interact* 1988;68:273–298. [PubMed: 3214888]
7. Yoon B, Hirabayashi Y, Kawasaki Y, Kodama Y, Kaneko T, Kanno J, Kim D, Fujii-Kuriyama Y, Inoue T. Aryl hydrocarbon receptor mediates benzene-induced hematotoxicity. *Toxicol Sci* 2002;70:150–156. [PubMed: 12388843]
8. Hirabayashi Y, Yoon B, Li GX, Fujii-Kuriyama Y, Kaneko T, Kanno J, Inoue T. Benzene-induced hematopoietic toxicity transmitted by AhR in wild-type mouse and nullified by repopulation with AhR-deficient bone marrow cells: Time after benzene treatment and recovery. *Chemosphere* 2008;73:S290–S294. [PubMed: 18514254]
9. Nebert DW, Roe AL, Dieter MZ, Solis WA, Yang Y, Dalton TP. Role of the aromatic hydrocarbon receptor and [Ah] gene battery in the oxidative stress response, cell cycle control, and apoptosis. *Biochem Pharmacol* 2000;59:65–85. [PubMed: 10605936]
10. Bauer AK, Faiola B, Abernethy DJ, Marchan R, Pluta LJ, Wong VA, Gonzalez FJ, Butterworth BE, Borghoff SJ, Everitt JI, Recio L. Male mice deficient in microsomal epoxide hydrolase are not susceptible to benzene-induced toxicity. *Toxicol Sci* 2003;72:201–209. [PubMed: 12655032]
11. Hirabayashi Y, Miyajima A, Yokota T, Arai KI, Li GX, Yoon BI, Kaneko T, Kanno J, Inoue T. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on B cell differentiation in mouse pre-B colonization model regulated by artificially introduced human IL-3 receptors. *Organohalogen Comp* 2002;55:359–362.
12. van Grevenynghe J, Bernard M, Langouet S, Le Berre C, Fest T, Fardel O. Human CD34-positive hematopoietic stem cells constitute targets for carcinogenic polycyclic aromatic hydrocarbons. *J Pharmacol Exp Ther* 2005;314:693–702. [PubMed: 15860575]
13. Singh KP, Wyman A, Casado FL, Garrett RW, Gasiewicz TA. Treatment of mice with the Ah receptor agonist and human carcinogen dioxin results in altered numbers and function of hematopoietic stem cells. *Carcinogenesis* 2009;30:11–19. [PubMed: 18820284]
14. Ivanova NB, Dimos JT, Schaniel C, Hackney JA, Moore KA, Lemischka IR. A stem cell molecular signature. *Science* 2002;298:601–604. [PubMed: 12228721]
15. Favreau LV, Pickett CB. Transcriptional regulation of the rat NAD(P)H:quinone reductase gene. Identification of regulatory elements controlling basal level expression and inducible expression by planar aromatic compounds and phenolic antioxidants. *J Biol Chem* 1991;266:4556–4561. [PubMed: 1900296]
16. Rushmore TH, Pickett CB. Transcriptional regulation of the rat glutathione S-transferase Ya subunit gene. *J Biol Chem* 1990;265:14648–14653. [PubMed: 2387873]
17. Recio L, Bauer A, Faiola B. Use of genetically modified mouse models to assess pathways of benzene-induced bone marrow cytotoxicity and genotoxicity. *Chem Biol Interact* 2005;153–154:159–164.
18. Lan Q, Zhang L, Li G, Vermeulen R, Weinberg RS, Dosemeci M, Rappaport SM, Shen M, Alter BP, Wu Y, Kopp W, Waidyanatha S, Rabkin C, Guo W, Chanock S, Hayes RB, Linet M, Kim S, Yin S, Rothman N, Smith MT. Hematotoxicity in workers exposed to low levels of benzene. *Science* 2004;306:1774–1776. [PubMed: 15576619]
19. Wan J, Shi J, Hui L, Wu D, Jin X, Zhao N, Huang W, Xia Z, Hu G. Association of genetic polymorphisms in CYP2E1, MPO, NQO1, GSTM1, and GSST1 genes with benzene poisoning. *Environ Health Perspect* 2002;110:1213–1218. [PubMed: 12460800]
20. Badham HJ, Winn LM. Investigating the role of the aryl hydrocarbon receptor in benzene-initiated toxicity in vitro. *Toxicology* 2007;229:177–185. [PubMed: 17161514]

21. Fernandez-Salguero P, Pineau T, Hilbert DM, McPhail T, Lee SS, Kimura S, Nebert DW, Rudikoff S, Ward JM, Gonzalez FJ. Immune system impairment and hepatic fibrosis in mice lacking the dioxin-binding Ah receptor. *Science* 1995;268:722–726. [PubMed: 7732381]
22. Schmidt JV, Su GH, Reddy JK, Simon MC, Bradfield CA. Characterization of a murine Ahr null allele: involvement of the Ah receptor in hepatic growth and development. *Proc Natl Acad Sci USA* 1996;93:6731–6736. [PubMed: 8692887]
23. Fernandez-Salguero PM, Ward JM, Sundberg JP, Gonzalez FJ. Lesions of aryl hydrocarbon receptor-deficient mice. *Vet Pathol* 1997;34:605–614. [PubMed: 9396142]
24. Lahvis GP, Pyzalski RW, Glover E, Pitot HC, McElwee MK, Bradfield CA. The aryl hydrocarbon receptor is required for developmental closure of the ductus venosus in the neonatal mouse. *Mol Pharmacol* 2005;67:714–720. [PubMed: 15590894]
25. McMillan BJ, Bradfield CA. The aryl hydrocarbon receptor sans xenobiotics: endogenous function in genetic model systems. *Mol Pharmacol* 2007;72:487–498. [PubMed: 17535977]
26. Quintana FJ, Basso AS, Iglesias AH, Korn T, Farez MF, Bettelli E, Caccamo M, Oukka M, Weiner HL. Control of T(reg) and T(H)17 cell differentiation by the aryl hydrocarbon receptor. *Nature* 2008;453:65–71. [PubMed: 18362915]
27. Velhoen M, Hirota K, Westendorf AM, Buer J, Dumoutier L, Renauld JC, Stockinger B. The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. *Nature* 2008;453:106–109. [PubMed: 18362914]
28. Kimura A, Naka T, Nohara K, Fujii-Kuriyama Y, Kishimoto T. Aryl hydrocarbon receptor regulates Stat1 activation and participates in the development of Th17 cells. *Proc Natl Acad Sci USA* 2008;105:9721–9726. [PubMed: 18607004]
29. Stevens EA, Mezrich JD, Bradfield CA. The aryl hydrocarbon receptor: a perspective on potential roles in the immune system. *Immunology* 2009;127:299–311. [PubMed: 19538249]
30. Head JL, Lawrence BP. The aryl hydrocarbon receptor is a modulator of anti-viral immunity. *Biochem Pharmacol* 2009;77:642–653. [PubMed: 19027719]
31. Shi LZ, Faith NG, Nakayama Y, Suresh M, Steinberg H, Czuprynski CJ. The aryl hydrocarbon receptor is required for optimal resistance to *Listeria monocytogenes* infection in mice. *J Immunol* 2007;179:65–71.
32. Fingerhut MA, Halperin WE, Marlow DA. Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *N Engl J Med* 1991;324:212–218. [PubMed: 1985242]
33. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans: Polychlorinated Dibenzo-Para-Dioxins and Polychlorinated Dibenzofurans, IARC Monogr. Eval Carcinog Risks Hum 1997;69:1–631.
34. Bertazzi PA, Pesatori AC, Bernucci I, Landt MT, Consonni C. Dioxin exposure and human leukemias and lymphomas. Lessons from the Seveso accident and studies on industrial workers. *Leukemia* 1999;133(Suppl 1):S72–S74. [PubMed: 10232369]
35. Baccarelli A, Morarelli P, Patterson DG Jr, Bonzini M, Pesatori AC, Caporaso N, Landt MT. Immunologic effects of dioxin: new results from Seveso and comparison to other studies. *Env Health Perspect* 2002;110:1169–1173. [PubMed: 12460794]
36. Zheng T, Blair A, Zhang Y, Weisenburger DD, Zahm A. Occupation and risks of non-Hodgkin's lymphoma and chronic lymphocytic leukemia. *J Occup Environ Med* 2002;44:469–474. [PubMed: 12024692]
37. Magnani C, Pastore G, Luzzatto L, Terracini B. Parental occupation and other environmental factors in the etiology of leukemias and non-Hodgkin's lymphoma in childhood: A case-control study. *Tumori* 1990;76:413–419. [PubMed: 2256184]
38. Filippini G, Farinotti M, Ferrarini M. Active and passive smoking during pregnancy and risk of central nervous system tumors in children. *Paediatr Perinat Epidemiol* 2000;14:78–84. [PubMed: 10703038]
39. Gebremichael A, Tullis K, Denison MS, Cheek JM, Pinkerton KE. Ah-receptor-dependent modulation of gene expression by aged and diluted sidestream cigarette smoke. *Toxicol Appl Pharmacol* 1996;141:76–83. [PubMed: 8917678]
40. Dertinger SD, Nazarenko DA, Silverstone AE, Gasiewicz TA. Aryl hydrocarbon receptor signaling plays a significant role in mediating benzo[a]pyrene- and cigarette smoke condensate-induced cytogenetic damage in vivo. *Carcinogenesis* 2001;22:171–177. [PubMed: 11159756]

41. Fine JS, Gasiewicz TA, Fiore NC, Silverstone AE. Prothymocyte activity is reduced by perinatal 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure. *J Pharmacol Exp Therap* 1990;255:128–132. [PubMed: 2213547]
42. Fine JS, Silverstone AE, Gasiewicz TA. Impairment of prothymocyte activity by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *J Immunol* 1990;144:1169–1176. [PubMed: 2303704]
43. Staples JE, Murante FG, Fiore NC, Gasiewicz TA, Silverstone AE. Thymic alterations induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin are strictly dependent on aryl hydrocarbon receptor activation in hemopoietic cells. *J Immunol* 1998;160:3844–3854. [PubMed: 9558089]
44. Laiosa MD, Wyman A, Murante FG, Fiore NC, Staples JE, Gasiewicz TA, Silverstone AE. Cell proliferation arrest within intrathymic lymphocyte progenitor cells causes thymic atrophy mediated by the aryl hydrocarbon receptor. *J Immunol* 2003;171:4582–4591. [PubMed: 14568932]
45. Thurmond TS, Gasiewicz TA. A single dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin produces a time- and dose-dependent alteration in the murine bone marrow B-lymphocyte maturation profile. *Toxicol Sci* 2000;58:88–95. [PubMed: 11053544]
46. Murante FG, Gasiewicz TA. Hemopoietic progenitor cells are sensitive targets of 2,3,7,8-tetrachlorodibenzo-p-dioxin in C57BL/6J mice. *Toxicol Sci* 2000;54:374–383. [PubMed: 10774819]
47. Garrett RW, Gasiewicz TA. The aryl hydrocarbon receptor agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin alters the circadian rhythms, quiescence, and expression of clock genes in murine hematopoietic stem and progenitor cells. *Mol Pharmacol* 2006;69:2076–2083. [PubMed: 16556773]
48. Sakai R, Kajiume T, Inoue H, Kanno R, Miyazaki M, Ninomiya Y, Kanno M. TCDD treatment eliminates the long-term reconstitution activity of hematopoietic stem cells. *Toxicol Sci* 2003;72:84–91. [PubMed: 12604837]
49. Fleming WH, Alpern EJ, Uchida N, Ikuta K, Spangrude GJ, Weissman IL. Functional heterogeneity is associated with the cell cycle status of murine hematopoietic stem cells. *J Cell Biol* 1993;122:897–902. [PubMed: 8349737]
50. Jetmore A, Plet PA, Tong X, Wolber FM, Breese R, Abonour R, Orschell-Traycoff CM, Srouf EF. Homing efficiency, cell cycle kinetics and survival of quiescent and cycling human CD34(+) cells transplanted into conditional NOD/SCID recipients. *Blood* 2002;99:1585–1593. [PubMed: 11861272]
51. Orschell-Traycoff CM, Hiatt K, Dagher RN, Rice S, Yoder MC, Srouf EF. Homing and engraftment potential of Sca-1+Lin- cells fractionated on the basis of adhesion molecule expression and position in cell cycle. *Blood* 2000;96:1380–1387. [PubMed: 10942381]
52. Gasiewicz TA, Henry EC, Collins LL. Expression and activity of aryl hydrocarbon receptors in development and cancer. *Crit Rev Eukaryotic Gene Exp* 2008;18:279–321.
53. Sartor MA, Schnekenburger M, Marlowe JL, Reichard JF, Wang Y, Fan Y, Ma C, Karyala S, Halbleib D, Liu X, Medvedovic M, Puga A. Genomewide analysis of aryl hydrocarbon receptor binding sites reveals an extensive array of gene clusters that control morphogenetic and developmental patterns. *Environ Health Perspect* 2009;117:1139–1146. [PubMed: 19654925]
54. Sugiyama T, Kohara H, Noda M, Nagasawa T. Maintenance of hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. *Immunity* 2006;25:977–988. [PubMed: 17174120]
55. Kim DW, Gazourian L, Quadri SA, Romieu-Mourez R, Sherr DH. The RelA NF-kappaB subunit and the aryl hydrocarbon receptor (AhR) cooperate to transactivate the c-myc promoter in mammary cells. *Oncogene* 2000;19:5498–5506. [PubMed: 11114727]
56. Thomsen JS, Keitz S, Strom A, Gustafsson J-A. HES-1, a novel target gene for the aryl hydrocarbon receptor. *Mol Pharmacol* 2004;65:165–171. [PubMed: 14722248]
57. Terskikh AV, Miyamoto T, Chang C, Diatchenko L, Weissman IL. Gene expression analysis of purified hematopoietic stem cells and committed progenitors. *Blood* 2003;102:94–101. [PubMed: 12623852]
58. Bruno L, Hoffman R, McBlane F, Brown J, Gupta R, Joshi C. Molecular signatures of self-renewal, differentiation, and lineage choice in multipotential hemopoietic progenitor cells in vitro. *Mol Cell Biol* 2004;24:741–756. [PubMed: 14701746]
59. Sang L, Coller HA, Roberts JM. Control of the reversibility of cellular quiescence by the transcriptional repressor HES1. *Science* 2008;321:1095–1100. [PubMed: 18719287]

60. Harper PA, Riddick DS, Okey AB. Regulating the regulator: factors that control levels and activity of the aryl hydrocarbon receptor. *Biochem Pharmacol* 2006;72:267–279. [PubMed: 16488401]
61. Mitchell KA, Elferink CJ. Timing is everything: Consequences of transient and sustained AhR activity. *Biochem Pharmacol* 2009;77:947–956. [PubMed: 19027718]
62. Pollenz RS. The aryl-hydrocarbon receptor, but not the aryl-hydrocarbon receptor nuclear translocator protein, is rapidly depleted in hepatic and nonhepatic culture cells exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Mol Pharmacol* 1996;49:391–398. [PubMed: 8643077]
63. Davarinos NA, Pollenz RS. Aryl hydrocarbon receptor imported into the nucleus following ligand binding is rapidly degraded via the cytoplasmic proteasome following nuclear export. *J Biol Chem* 1999;274:28708–28715. [PubMed: 10497241]
64. Roberts BJ, Whitelaw ML. Degradation of the basic helix-loop-helix/Per-ARNT-Sim homology domain dioxin receptor via the ubiquitin/proteasome pathway. *J Biol Chem* 1999;274:36351–36356. [PubMed: 10593927]
65. Mimura J, Ema M, Sogawa K, Fujii-Kuriyama Y. Identification of a novel mechanism of regulation of Ah (dioxin) receptor function. *Genes and Develop* 1999;13:20–25. [PubMed: 9887096]
66. Evans BR, Karchner SI, Allan LL, Pollenz RS, Tanguay RL, Jenny MJ, Sherr DH, Hahn ME. Repression of aryl hydrocarbon receptor (AHR) signaling by AHR repressor: role of DNA binding and competition for AHR nuclear translocator. *Mol Pharmacol* 2008;73:387–398.
67. Hesterman EV, Stegeman JJ, Hahn ME. Relative contributions of affinity and intrinsic efficacy to aryl hydrocarbon receptor ligand potency. *Toxicol Appl Pharmacol* 2000;168:160–172. [PubMed: 11032772]
68. Henry EC, Gasiewicz TA. Agonist but not antagonist ligands induce conformational changes in the mouse aryl hydrocarbon receptor as detected by partial proteolysis. *Mol Pharmacol* 2003;63:392–400. [PubMed: 12527811]
69. Venezia TA, Merchant AA, Ramos CA, Whitehouse NL, Young AS, Shaw CA, Goodell MA. Molecular signatures of proliferation and quiescence in hematopoietic stem cells. *PLOS Biol* 2004;2:1640–1651.
70. Noda S, Horiguchi K, Ichikawa H, Miyoshi H. Repopulating activity of ex vivo-expanded murine hematopoietic stem cell residues in the CD48-c-kit+Sca-1+ lineage marker- cell population. *Stem Cells* 2008;26:646–655. [PubMed: 18079432]
71. Mulero-Navarro S, Carvajal-Gonzalez JM, Herranz M, Ballesar E, Fraga MF, Ropero S, Esteller M, Fernandez-Salguero PM. The dioxin receptor is silenced by promoter hypermethylation in human acute lymphoblastic leukemia through inhibition of Sp1 binding. *Carcinogenesis* 2006;27:1099–1104. [PubMed: 16410262]
72. de Haan G, Nijhof W, Van Zant G. Mouse strain-dependent changes in frequency and proliferation of hematopoietic stem cells during aging: correlation between lifespan and cycling activity. *Blood* 1997;89:1543–1550. [PubMed: 9057635]
73. Sudo K, Ema H, Morita Y, Nakauchi H. Age-associated characteristics of murine hematopoietic stem cells. *J Exp Med* 2000;192:1273–1280. [PubMed: 11067876]
74. de Haan G, Van Zant G. Genetic analysis of hemopoetic cell cycling in mice suggests its involvement in organismal life span. *FASEB J* 1999;13:707–713. [PubMed: 10094931]
75. Nebert DW, Brown DD, Towne DW, Eisen HJ. Association of fertility, fitness, and longevity with the murine Ah locus among (C57BL/6N) (C3H/HeN) recombinant inbred strains. *Biol Reprod* 1984;30:363–373. [PubMed: 6704471]
76. Spindler SR, Koizumi A, Walford RL, Mote PL. P1-450 and P3-450 gene expression and maximum life span in mice. *Mut Res* 1996;219:1011–1016.
77. de Haan G, Van Zant G. Dynamic changes in mouse hematopoietic stem cell numbers during aging. *Blood* 1999;93:790–7296.
78. Morrison SJ, Wandycz AM, Akashi K, Globerson A, Weissman IL. The aging of hematopoietic stem cells. *Nature Med* 1996;2:1011–1016. [PubMed: 8782459]
79. Chen J. Senescence and functional failure in hematopoietic stem cells. *Exp Hematol* 2004;32:1025–1032. [PubMed: 15539079]
80. Kim M, Moon HB, Spangrude GJ. Major age-related changes of mouse hematopoietic stem/progenitor cells. *Ann NY Acad Sci* 2003;996:195–208. [PubMed: 12799297]

81. Van Zant G, Liang Y. The role of stem cells in aging. *Exp Hematol* 2003;31:659–672. [PubMed: 12901970]
82. Bell DR, Van Zant G. Stem cells, aging and cancer: inevitabilities and outcomes. *Oncogene* 2004;23:7290–7296. [PubMed: 15378089]
83. Morse HC III, Anver MR, Fredrickson TN, Haines DC, Harris AW, Harris NL, Jaffe ES, Kogan SC, MacLennan ICM, Pettengale PK, Ward JM. Bethesda proposals for classification of lymphoid neoplasms in mice. *Blood* 2002;100:246–258. [PubMed: 12070034]
84. Karsunky H, Zeng H, Schmidt T, Zevnik B, Kluge R, Shmid KW, Duhrsen U, Moroy T. Inflammatory reactions and severe neutropenia in mice lacking the transcriptional repressor Gfi1. *Nature Genet* 2002;30:295–300. [PubMed: 11810106]
85. Shimizu R, Kuroha T, Ohneda O, Pan X, Ohneda K, Takahashi S, Philipsen S, Yamamoto M. Leukemogenesis caused by incapacitated GATA-1 function. *Mol Cell Biol* 2004;24:10814–10825. [PubMed: 15572684]
86. Jacob B, Osato M. Stem cell exhaustion and leukemogenesis. *J Cell Biochem* 2009;107:393–399. [PubMed: 19347871]
87. Hirabayashi Y, Yoon BI, Fujii-Kuriyama Y, Kaneko T, Kanno J, Inoue T. Tumor suppressor function of aryl hydrocarbon receptor (AhR): Early onset of spontaneous lymphoma and hepatoma, and resulting shortened life span observed in AhR deficient mice. *Organohal Comp* 2006;68:556–559.

Table 1

Evidence That The Xenobiotic AhR Ligand TCDD Affects Hematopoiesis in Mice

Hematopoietic Endpoint	Reference
Reduced thymic seeding by bone marrow progenitors	[41,42]
Decreased terminal deoxynucleotidyl transferase (TdT) and recombina- activating gene-1 (RAG-1) mRNA in bone marrow	[41,42]
Required AhR presence in hematopoietic cells for effects on altered thymic seeding and TdT/RAG-1	[43,44]
Decreased number of immature B cells in bone marrow; decreased number of CFU-pre-B progenitors	[45]
Increased number of HSC-enriched Lin-/Sca-1 ⁺ /c-kit ⁺ (LSK) cells, also being dependent on AhR in hematopoietic cells	[43,46]
Altered circadian rhythms, percentage of cells in quiescence, and gene expression	[47]
Skewed differentiation from lymphoid lineage in favor of granulocyte/macrophage lineage	[13]
Decreased engraftment of HSCs into lethally- irradiated mice	[13,48]

TABLE 2**Mechanisms Controlling AhR Expression and Activity**

Mechanism	References
Proteosomal degradation of AhR protein	[62–64]
Presence and induction of the AhR Repressor	[65,66]
Binding by agonists vs antagonists	[67,68]
Metabolism of AhR ligands	[61,69,70]
Transient vs sustained AhR activity	[61]
Regulation of the Ahr gene by other transcription factors	[60]