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REVIEW

The role of the aryl hydrocarbon receptor in normal and malignant B cell development

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Abstract The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor historically studied for its role in environmental chemical-mediated toxicity and carcinogenicity. In the last 5 years, however, it has become clear that the AhR, presumably activated by endogenous ligand(s), plays an important role in immune system development and function. Other articles in this edition summarize AhR function during T cell and antigen-presenting cell development and function, including the effects of AhR activation on dendritic cell function, T cell skewing, inflammation, and autoimmune disease. Here, we focus on AhR expression and function during B cell differentiation. Studies exploiting immunosuppressive environmental chemicals to probe the role of the AhR in humoral immunity are also reviewed to illustrate the multiple levels at which a "nominally activated" AhR could control B cell differentiation from the hematopoietic stem cell through the pro-B cell, mature B cell, and antibody-secreting plasma cell stages. Finally, a putative role for the AhR in the basic biology of B cell malignancies, many of which have been associated with exposure to environmental AhR ligands, is discussed.

Keywords Aryl hydrocarbon receptor · B cell development · Lymphoma · Myeloma

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Introduction

The study of the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor, has come a long way. For many years, analysis of AhR function and activity was the purview solely of toxicologists interested in understanding how environmental chemicals are "sensed" by biological organisms. With regard to the immune system in particular, immunotoxicologists focused on a set of environmentally common, immunosuppressive chemicals including dioxins, most notably 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs). These studies, in essence, exploited environmental chemicals as probes of biological systems to understand how the AhR functions and to begin to reveal for what purpose this evolutionarily conserved receptor/transcription factor exists. These studies were of enormous value since they provided a scaffold on which to build theories of the "nominal" function of the AhR. They also suggested that, at various points in B cell development and differentiation, B cells themselves, or stromal cells on which B cells depend for developmental signals, express AhR and serve as the immediate targets of endogenous or exogenous AhR ligands. Indeed, analysis of gene expression profiles in a panel of purified, developmentally defined normal murine B cells [1] demonstrates a hierarchy of AhR expression during B cell development (Fig. 1). Bone marrow pro- and pre-B cells express little or no *AhR* mRNA. In contrast, splenic transitional B cells, representing cells recently activated during clonal selection, have elevated *AhR* levels. Follicular, marginal zone, or germinal center B cells and plasmablasts express modest but variable *AhR* levels. Interestingly, plasma cells express high AhR levels, suggesting a role for the AhR in plasma cell development and/or function. This issue will be readdressed later in this manuscript.

Here, we summarize representative studies that illustrate the multiple levels at which the AhR may contribute to B cell development and function. We begin with early studies that employed either in vivo models or complex in vitro systems

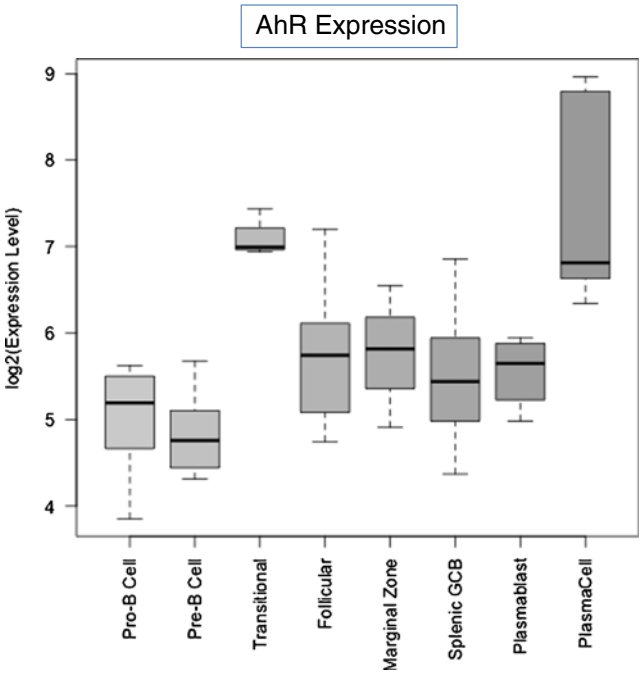


Fig. 1 Relative *AhR* mRNA expression in purified subpopulations of murine B cells. Microarray data were generated by Green et al. [1] from murine B cells sorted by flow cytometry based on B developmental stage-specific surface antigens. Expression levels of the AhR transcript within the listed differentiation stages was extracted from [1] and the corresponding distributions summarized and displayed as 'box-and-whiskers' plots (with the bottom and the top of the box corresponding to the first and third quartiles, the thick band inside the box indicating the median, and the end of the 'whiskers' extending to 1.5 times the interquartile range in both directions)

consisting of cocultures of B cells and cells from the lymphoid microenvironment. We continue with models using B cell lines or purified B cells to assess the role of the AhR in activated B cell and plasma cell differentiation and in antibody production and conclude with a discussion of the likely role of the AhR in B cell malignancies.

Environmental chemicals as probes for AhR control of B cell development and function in vivo or in complex organ culture systems

Early studies that evaluated the mechanisms by which dioxins, PAHs, and PCBs mediate immunosuppression demonstrated that these environmental AhR ligands suppress immunity by compromising virtually every stage of lymphocyte development, activation, and effector function, implying that the AhR plays an important role at several levels of B cell differentiation. For example, halogenated aromatic hydrocarbons were shown to suppress B and T cell development in primary lymphoid organs and to compromise antibody responses in vivo and in organ cultures [2–9]. TCDD, the quintessential high-affinity AhR ligand, was shown to be particularly immunosuppressive, significantly inhibiting lymphocyte development in vivo at doses in the nanograms per kilogram range [10]. For example, administration of 10–100 ng/kg TCDD resulted in a significant increase in mortality after influenza virus infection [11–14] and weakened memory responses [15]. At slightly higher doses (100–1,000 ng/kg), TCDD induced thymic atrophy [16–18], reduced resistance to parasites [19], and suppressed humoral responses in an AhR-dependent fashion [9, 20, 21]. The ability of AhR binding, but not nonbinding TCDD congeners, to affect the suppression of B cell responses and the relative resistance of lymphocytes generated from mice expressing low-affinity AhR (AhR^d) to TCDD [19] confirmed that TCDD-induced inhibition of humoral responses is AhR mediated.

PAHs and PCBs were also shown to suppress B cell responses in vivo, although likely through somewhat different mechanisms than TCDD. For example, the prototypic PAHs and AhR ligands, 7,12-dimethylbenz[a]anthracene and benzo[a]pyrene (B[a]P), suppressed in vivo humoral immune responses, and this immunosuppression was blocked by the addition of the partial AhR agonist/AhR inhibitor α-naphthoflavone [22, 23]. PAHs also decreased splenic B lymphocyte numbers and reduced the number of antigen-specific effector B cells [24–26].

Similarly, the halogenated hydrocarbons, 3,3′,4,4′-tetrachlorobiphenyl and 2,3,3′,4,4′,5-hexachlorobiphenyl, suppressed antibody responses to challenge with lipopolysaccharide (LPS) or sheep red blood cells [27, 28]. At least some of this apparent suppression of B cell differentiation induced by PAHs and PCBs was dependent on the degree to which the AhR ligand could be metabolized [22, 23, 29]. This conclusion still has relevance to AhR function, since the activation of the AhR regulates the transcription of a battery of P450 genes (*CYP1A1*, *CYP1A2*, and *CYP1B1*) critical to oxidative, phase I metabolism of environmental or endogenous compounds [30, 31]. Furthermore, the fact that all of these outcomes were AhR dependent suggested that the AhR is expressed in multiple components of the immune system and, therefore, is likely to play a role in the development of mature B cell responses.

Since developing biological systems are more sensitive to environmental stressors than mature systems, one might predict that developing B cells would be more sensitive to AhR ligands than mature, antibody-secreting B lineage cells. Model systems of B cell development involving cultures of bone marrow cells containing both B lineage cells and bone marrow stromal cells (e.g., Whitlock/Witte cultures) were used to test this hypothesis. Since bone marrow B cells are poised to undergo clonal deletion in response to self-antigen [32, 33], it was predicted that these cells would have a low threshold of apoptosis induction in response to AhR ligands. Indeed, in long-term Whitlock/Witte cultures of primary bone marrow cells, relatively low PAH doses (10 nM) rapidly induced apoptosis in $B220^+/CD43^-/sIg^-$ pre-B cells (B cell fraction D) or in a bone marrow–stromal cell-

dependent B220$^+$/CD43$^+$ primary pro/pre-B cell line (BU-11) (B cell fraction B/C) [4, 5]. That the AhR was required for PAH-mediated apoptosis induction was supported by the ability of α-naphthoflavone or galangin, two partial AhR agonists (effective antagonists in the presence of a higher-affinity AhR ligand), to block apoptosis [4, 34]. Studies on apoptosis signaling pathways indicated: (1) PAH-induced downregulation of the anti-apoptotic NF-κB subunits Rel A and c-Rel as well as the anti-apoptotic NF-κB gene target c-*myc* [35, 36], (2) robust induction of the intrinsic apoptosis pathway involving cytochrome *c* release from mitochondria but not a mitochondrial membrane depolarization, (3) activation of APAF1 and formation of the apoptosome, (4) triggering of a caspase-8-dependent positive feedback loop, and (5) activation of executioner caspase-3 [37–39]. Interestingly, physical contact between the culture dish-adherent bone marrow stromal cells and stromal cell-adherent pro/pre-B cells was required for PAH-induced apoptosis [38], and treatment of pro/pre-B cells in the absence of stromal cells but in the presence of supportive IL-7 failed to induce B cell death [5, 40]. These results indicated that pro/pre-B cell apoptosis is the result of a "gain of function" as opposed to, for example, the loss of the production by stromal cells of cytokines critical to B cell survival. Surprisingly, murine bone marrow stromal cells, but not pro- or pre-B cells, were shown to express AhR [4, 5]. These data are consistent with microarray results in which little or no *AhR* mRNA was detected at the pro- or pre-B cell stage (Fig. 1). Furthermore, PAHs induced bone marrow B cell apoptosis in cocultures of primary stromal cells from AhR$^{+/+}$ but not from AhR$^{-/-}$ mice [40]. These results indicate that stromal cells are a direct target of PAH and suggest that stromal cells deliver a cell contact-dependent "death signal" to adjacent pro/pre-B cells. Since bone marrow stromal cells express a functional AhR and since several hematopoietic cell types depend on these cells for growth and differentiation signals, it seems likely that AhR activity in the bone marrow microenvironment contributes, in an as yet undetermined manner, to the development of several hematopoietic lineages. AhR expression in stromal cells also suggests the possibility that aberrant AhR activation in the bone marrow microenvironment, for example by environmental ligands, contributes to the development of B cell malignancies that originate in the bone marrow, e.g., multiple myeloma, a disease already associated with exposure to environmental AhR ligands (see the succeeding paragraphs).

The failure of poorly metabolized AhR ligands to induce pre-B cell apoptosis [41], and a requirement for AhR-dependent CYP1B1 expression in bone marrow stromal cells for PAH-induced bone marrow B cell apoptosis [42, 43], indicated that AhR activation alone is not sufficient to induce B cell apoptosis and suggested that AhR-regulated PAH metabolism is required for the induction of an apoptosis signal. Indeed, the addition of PAH metabolites to bone marrow cultures obviated the need for AhR$^+$ stromal cells for apoptosis induction in bone marrow B cells [41, 43]. Interestingly, stromal cell-derived PAH metabolites were shown to be transferred from the stromal cells to stromal cell-adherent bone marrow B cells by a unique mechanism, i.e., exchange of membranes between the two cell types (trogocytosis) [38]. The predicted sensitivity of developing B cells was supported by the failure of bone marrow stromal cells to exchange membranes with or to induce apoptosis in mature B cells or T cells, neither of which undergo apoptosis in response to PAH treatment even in the presence of stromal cells [38]. Collectively, these studies emphasize the dependence of early B cells on their stromal microenvironment and indicate that the AhR may control early B cell development indirectly by altering the bone marrow milieu. The demonstration of aberrations in bone marrow B cell development in AhR$^{-/-}$ mice is consistent with this model [8].

A note of caution is required in interpreting experiments with exogenous sources of AhR ligands. It is well established that different AhR ligands induce different outcomes in a tissue-specific and context-specific manner. Outcomes with TCDD may or may not exactly replicate outcomes with endogenous AhR ligands. Therefore, studies utilizing any surrogate AhR ligand can only demonstrate the presence of a functional AhR and suggest, but not prove, the nature of the AhR response to other ligands, including the response to endogenous ligands made either by the B lineage cell itself or its microenvironment.

Environmental chemicals as probes for AhR control of B cell development and function in clonal or purified B cell model systems

While the studies described previously were important for assessing AhR-mediated events in vivo or in systems designed to model interactions between developing B cells and their microenvironment, they did not determine if AhR ligands directly affect AhR$^+$ mature B cells and, by inference, if the AhR plays a significant role in intracellular B cell signaling. In this vein, purified peripheral human B cells were isolated to assess AhR expression levels and to determine if mature B cells are affected by AhR engagement. Consistent with microarray data from purified murine B cell populations (Fig. 1), resting human B cells were shown to express relatively low AhR levels [44]. However, activation with CpG or CD40 ligand, surrogates for stimuli invoked during innate and adaptive immune responses, respectively, profoundly upregulated AhR mRNA and protein [44]. IL-4 treatment alone induced AhR expression in both murine and human B cells through a STA6-dependent pathway [45]. LPS or PMA+ionophore activation of murine splenic B cells similarly increased AhR and ARNT expression [46] and

rendered activated B cells more sensitive to AhR ligands than resting B cells [19, 47, 48]. AhR nuclear translocation, constitutive DNA binding, and induction of the AhR target gene *CYP1A1* in CpG-stimulated or CD40 ligand-stimulated human B cells [44], in PMA+ionophore-induced, and in IL-4-activated murine splenic B cells [47] suggested the presence of endogenous AhR ligands that drive AhR signaling in cultures of activated B cells. Inhibition of AhR activity by ectopic expression of an AhR repressor-encoding gene (*AhRR*) inhibited the proliferation of CpG-activated or CD40 ligand-activated human B cells (unpublished), suggesting that the AhR contributes to activated B cell proliferation. These results are consistent with those obtained in $AhR^{-/-}$ mice in which deficiencies in the accumulation of mature splenic lymphocytes, as well as peritoneal $CD5^+$ B-1 cells, were noted [49]. A role for the AhR in B cell growth is consistent with studies performed with other cell types in which constitutively active AhR was shown to regulate the cell cycle [50–52]. In this context, "constitutively active" is operationally defined as AhR continuously activated by endogenous ligand(s). Dimerization of the AhR with Rb, E2F [53–56], Rel A [57, 58], or Rel B [59] suggests some mechanisms through which the AhR could influence B cell growth. These findings also suggest a possible role for the AhR in regulating apoptosis in B cells. Indeed, at least one study demonstrated that transformed human B lymphoma cells undergo apoptosis on exposure to PAH [60]. Other studies have implicated the AhR in the control of apoptosis in other cell types [61–64].

Microarray studies demonstrate relatively high AhR levels in murine plasma cells (Fig. 1). In order to determine if the AhR plays a role in the differentiation of human B cells into plasma cells, we developed an in vitro system in which up to 40 % of CD40 ligand-activated, AhR^{high} human B cells could be induced to differentiate into plasma cells in the absence of feeder cells [65]. Since differentiation could be induced even when cell growth was blocked with low-level irradiation, the effects of AhR activation on differentiation and cell growth could be separated. In this system, AhR hyperactivation with B[a]P, a prototypic environmental PAH/AhR ligand, significantly blocked CD40 ligand-driven and cytokine-driven differentiation into $CD138^+$ plasma cells in the presence or absence of cell growth without affecting cell viability [65]. As with the cocultures of bone marrow stromal cells and pre- or pro/pre-B cells, AhR-regulated metabolism of the parent PAH was required for the inhibition of plasma cell formation.

The contribution of the AhR to antibody secretion was studied extensively in a $CD5^+$ murine B cell lymphoma line (CH12.LX). In early studies, it was shown that hyperactivation of the AhR with TCDD alters the ability of these AhR^+ cells to produce antibody in response to LPS by binding to and inhibiting the transcription of the 3′ alpha immunoglobulin heavy chain gene [66–71]. Inhibition of antibody production was not seen in AhR^- BCL-1 B cells [72]. AP1 (c-Jun), a component of the LPS-activated TLR-4 signaling pathway [73], also appeared to be targeted since its expression and function were downregulated by TCDD in AhR^+ CH12.LX, but not in AhR^- BCL-1, cells [72]. Follow-up studies further implicated the AhR in B cell differentiation by demonstrating that hyperactivated AhR decreases Blimp-1 expression and binding to the *PAX5* gene promoter [66]. The decrease in *Prdm1* (Blimp-1) transcription appears to be tied to the aforementioned decrease in AP1 since TCDD treatment decreased AP1 binding to *Prdm1* [66]. *Prdm1* transrepression may also be linked to an AhR-dependent transactivation of *Bach2*, a *Prdm1* repressor [74]. That is, TCDD-activated AhR binds to a cognate site in the first intron of *Bach2*, increasing Bach2 expression and binding to the Maf elements in *Prdm1* [74]. This circuit of transcription factors controlling B/plasma cell differentiation is summarized in Fig. 2.

These comprehensive studies illustrate the intersection of AhR signaling with several interconnecting pathways of B cell differentiation and effector function. Apropos of this, it has been postulated [75] that the AhR could alter B cell function by inhibiting antibody production to a level dictated by the degree of AhR activation and/or by interference with the "bi-stable switch circuit" involving Bcl6, Blimp-1, PAX5, and Bach2 and known to control an all-or-none cellular decision to differentiate from activated B cell into antibody-secreting plasma cell [76, 77] (Fig. 2). The demonstration that the AhR directly binds to the 3′ alpha Ig heavy chain promoter [66–71] suggests a mechanism for the former possibility, while AhR control of *AP1* [72], *Prdm1* [66], and *Bach2* transcription [74] is consistent with the latter. To distinguish between these two, non-mutually exclusive models, Zhang et al. performed an elegant study in which the propensity for LPS to induce antibody production and/or an all-or-none commitment to generating plasma cells in the

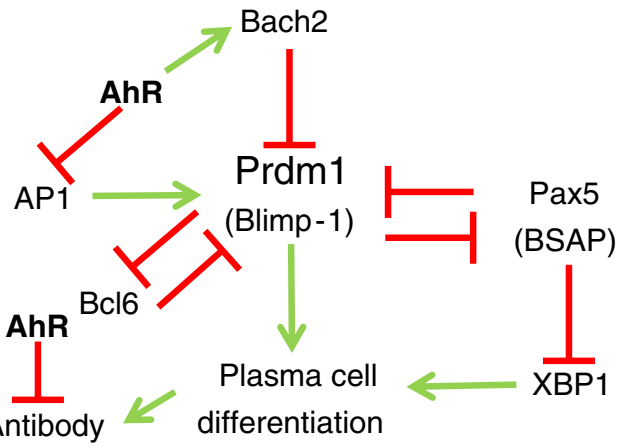


Fig. 2 AhR interactions with B cell differentiation decisions. A "bi-stable circuit" controls the decision of activated B cells to differentiate into antibody-secreting plasma cells. The consensus "all-or-none" switching pathway involves five interacting transcription factors: Prdm (encoding Blimp-1), AP1, Bach2, Pax5 (BSAP), Bcl6, and XBP1. The AhR has been shown to suppress the transcription of *AP1*, *Prdm1*, and *IgM* while enhancing the transcription of *Bach2*

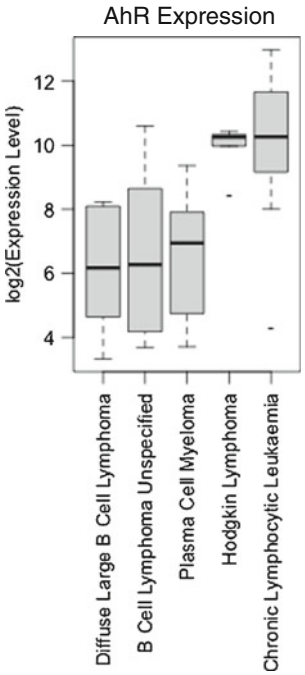


Fig. 3 *AhR* mRNA expression varies among five human B lineage cancers. Relative levels of AhR expression in five human B lineage cancers were assessed by analysis of microarray data obtained from 1,036 human cancer cell lines (http://www.broadinstitute.org/ccle/home). Each box plot reports the distribution of the AhR transcript within the samples belonging to the corresponding B lineage cancer type

presence or absence of TCDD was evaluated [75]. Using a combination of computational biology and flow cytometric analysis of LPS-induced antibody production and B cell terminal differentiation, it was concluded that TCDD-induced suppression of the IgM response occurs as a binary function, i.e., TCDD reduces the number of IgM-secreting cells in a dose-dependent manner in an "all-or-none" response rather than by proportionally decreasing the amount of antibody produced by any given plasma cell [75].

A more global analysis of potential targets of the AhR in TCDD-treated, LPS-activated CH12LX B lymphoma cells revealed a number of potential interactions between the AhR and genes critical to B cell development and function [78]. In this study, 1,893 regions, 1,035 of which mapped to within 10 kb of a known gene, exhibited increased AhR binding after TCDD treatment. These identified regions were then compared to a gene expression profile in which 422 genes exhibited increased expression 8 h after AhR activation. Seventy-eight of the upregulated genes were also contained in the set of 1,035 regions immunoprecipitated with the AhR, suggesting direct interactions between the AhR and these genes. Several of these genes are known to be critical to B cell development or function and contain multiple consensus AhR binding sites (5′-TNGCGTG-3′) within 3,000 bp upstream and 299 bp downstream of their respective gene start sites. (AhR binding sites are known to be located further upstream than is generally considered to be part of a prototypical gene promoter [79, 80]). For example, c-*myc* (seven consensus AhREs) plays a critical role in normal B cell growth [81] and has previously been shown to be directly regulated by the AhR in human mammary tumor cells [57, 82]. *Runx2* and *Runx3* (eight and seven AhREs, respectively), although most frequently associated with osteoblast [83] and T cell development [84], respectively, also appear to be involved in memory B cell formation [85]. XBP1 (four AhREs), repressed by Pax5 (nine AhREs), plays a critical role in plasma cell differentiation [86]. Finally, *SOCS3* (five AhREs) controls the response of B cells and plasma cells to STAT-3-dependent cytokines, including IL-21 and IL-6, and is critical to the formation of germinal centers [87]. Therefore, there is the potential for the AhR, activated by as yet unidentified endogenous ligands, to influence the transcription of several master regulators of B cell development.

AhR control of hematopoietic stem cell (HSC) development

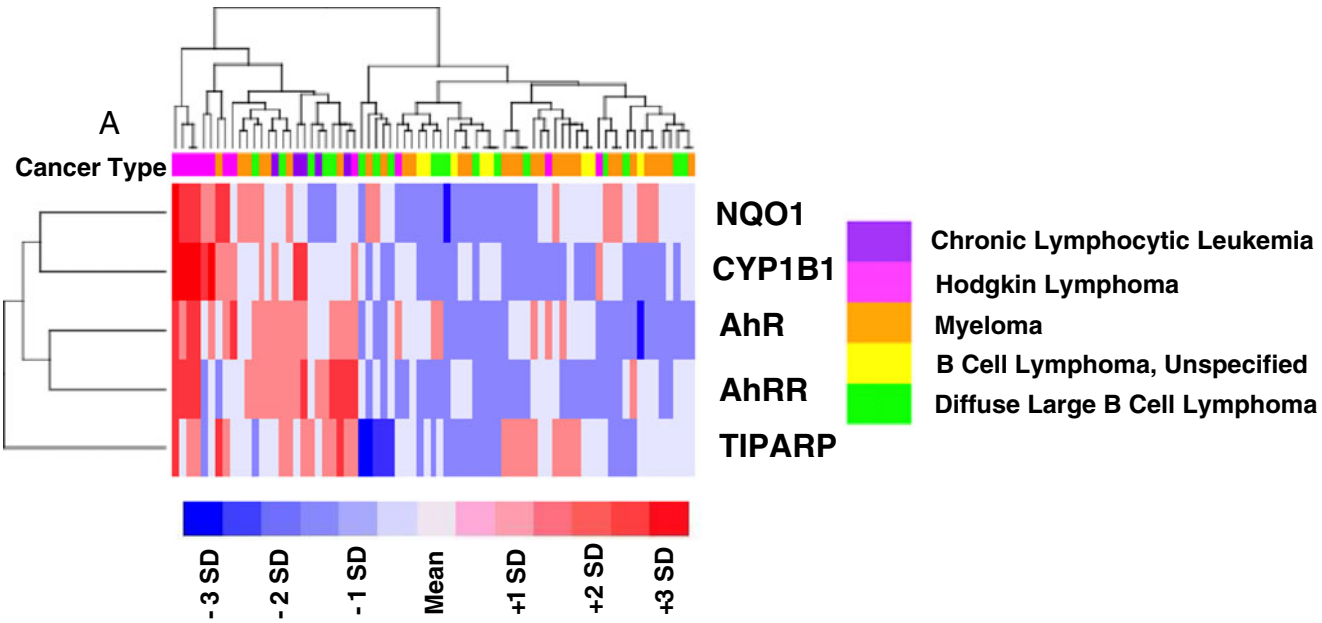
The differentiation of HSCs into all eight blood cell lineages is a tightly regulated process [88] that changes in subtle but important ways during life [89–92]. Disruption of this regulation has a profound downstream effect on multiple hematopoietic cell types, including B cells, leading to mixed lineage leukemias [93], lymphomas [94], stem cell exhaustion [95], and other blood cell disorders [96, 97]. Therefore, any role that the AhR plays in HSC differentiation will have a bearing on B cell development. Several studies indicate that murine and human HSC express modest AhR levels [98–100] and recent breakthrough studies indicate that the AhR plays a critical role in HSC growth and differentiation [95, 101–105]. For example, in vivo AhR modulation disrupts HSC growth, senescence, and migration [95, 101–103, 106, 107]. Of note is that most of these studies used environmental AhR ligands as probes to establish the nominal function of the AhR in HSCs [101, 102, 104, 106, 107]. Furthermore, $AhR^{-/-}$ mice exhibit an increased number of bone marrow HSCs [95] and pro/pre-B cells [8]. Perhaps most dramatically, AhR inhibition promotes the expansion of purified, human HSC, suggesting the use of AhR inhibitors as a clinical method for expanding HSC populations prior to stem cell transplant [105].

The AhR in B cell malignancies

The studies summarized previously demonstrate that the AhR plays an integral role in B cell development, likely through controlling cell growth and apoptosis. In other types of cells, the AhR regulates cell migration potentially through Slug, Vav3, TGF-β, and/or c-Jun [108–113]. Since dysregulated cell growth, apoptosis, and migration are hallmarks of cancer, it could

reasonably be hypothesized that aberrant AhR expression or activity could contribute to B cell malignancies. One prediction from this hypothesis would be an association between exposure to environmental AhR ligands and the risk of B lymphomas, leukemias, or multiple myeloma. Consistent with this prediction, exposure to TCDD, halogenated hydrocarbon-containing organochlorine pesticides, or PCBs significantly increases the risk of non-Hodgkin's lymphoma (NHL) [114–116]. In perhaps the best documented study of TCDD exposure which occurred following an explosion at an herbicide manufacturing facility in Seveso, Italy, a significant increase in the risk of NHL was documented in inhabitants of the surrounding communities (relative odds ratio=4.45) [117]. In addition, human AhR polymorphisms have been linked to the risk of NHL following exposure to organochlorines, some of which are AhR ligands [118]. Similarly, the risk of multiple myeloma in the TCDD-exposed Seveso population was significantly elevated, with a relative risk of 3.07 [117, 119]. Although controversial, a link between multiple myeloma risk in Vietnam War veterans exposed to the TCDD-contaminated defoliant, Agent Orange, has been suggested by a National Academy of Sciences review committee (http://books.nap.edu/openbook.php?record_id=13166). In interpreting these studies, it is important to note that TCDD, which exhibits a biological half-life of 7–11 years in humans, is a known human carcinogen but is not genotoxic, i.e., it does not directly induce mutations. Thus, its carcinogenicity likely reflects, at least in part, persistent AhR signaling.

In a fashion analogous to the analysis of immune modulation by environmental AhR ligands to elucidate AhR function during normal B cell development, analysis of cancer



B

Gene ID	Pearson Score	p value	FDR
AhR	1	0.002	0.0366
CYP1B1	0.5874	0.002	0.0366
TIPARP	0.5358	0.002	0.0366
AHRR	0.4637	0.002	0.0366
NQO1	0.1747	0.138	0.329

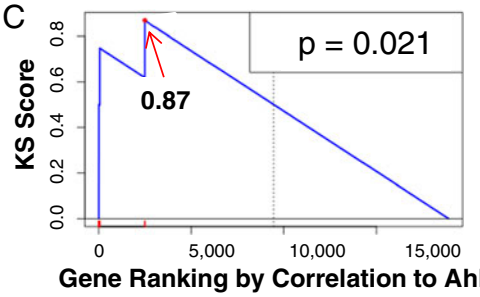


Fig. 4 Relative expression of *AhR* mRNA and AhR target genes in human tumor cell lines. Analysis of microarray data obtained from 1,036 human cancer cell lines (http://www.broadinstitute.org/ccle/home) is presented. **a** Data corresponding to the five lymphoid malignancies listed and five transcripts, including AhR and four of its putative targets, are displayed as a color-coded gene-by-sample heat map, with *rows* (genes) and *columns* (samples) sorted by hierarchical clustering [136]. **b** Microarray data for the same five lymphoid cancers were analyzed, and genes ranked by Pearson correlation between the level of AhR expression and that of four known AhR target genes, *CYP1B1*, *NQO1*, *TIPARP*, and *AhRR*. Permutation-based *p* values and the corresponding FDR-corrected *q* values are shown. **c** A Kolmogorov–Smirnov test was performed to assess the strength of the association between AhR and its four targets. The *x*-axis lists the genes in the human transcriptome sorted by their distance from AhR (from the closest, *left*, to the furthest, *right*). The position of the four AhR targets (*red ticks*) is significantly skewed toward the left-hand side of the list (permutation-based *p* value=0.021) [137]

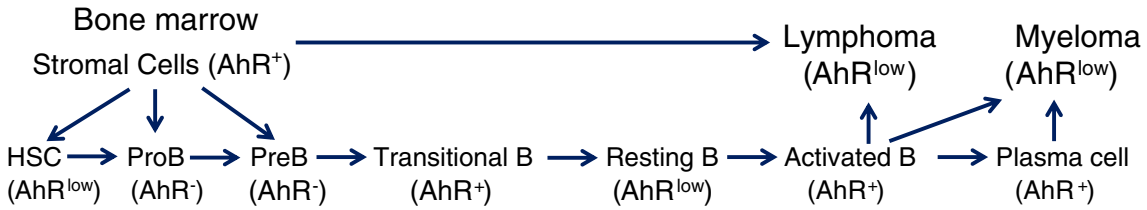


Fig. 5 Role of the AhR in the development of normal and malignant B cells. AhR is expressed variably during B cell differentiation. While HSC express low AhR levels, AhR expression is lost by the pro-B cell stage. Pre-B cells are similarly AhR^-. Transitional splenic B cells, which may have been recently activated by low-affinity autoantigens [138], upregulate AhR expression. Following clonal selection, resting B cells express little or no AhR. AhR is again upregulated on activation by foreign antigens with T cell help and during differentiation into plasma cells. AhR^+ bone marrow stromal cells facilitate normal bone marrow B cell growth and differentiation and likely play a critical role in supporting B lineage malignancies including lymphomas and multiple myelomas

induction with environmental AhR ligands pointed to a general role for the AhR in malignant B cell transformation. That the AhR plays a role in B cell malignancy regardless of cancer etiology was supported by many studies demonstrating elevated AhR levels and "constitutive" activity in a variety of cancer cell lines including lymphomas, myelomas, and T cell leukemias [50, 82, 120–126].

Microarray analysis of 1,036 human cancer cell lines generated at the Broad Institute, i.e., the Cancer Cell Line Encyclopedia (http://www.broadinstitute.org/ccle/home) (Fig. 3), revealed a hierarchy of AhR expression in which low levels of AhR were expressed in diffuse large B cell lymphomas, unspecified B cell lymphomas, and myelomas and notably higher levels in Hodgkin's lymphomas and chronic lymphocytic leukemias (Fig. 4). Interestingly, expression of three (*CYP1B1*, *TIPARP*, and *AhRR*) of four (*CYP1B1*, *TIPARP*, *AhRR*, and *NQO1*) well-established AhR target genes chosen at random appear to track with AhR expression in the B lineage cancer subtypes, suggesting, but not proving, constitutive AhR activity in these tumor lines as previously documented in other tumor types [50, 82, 120–126].

Although the molecular mechanisms through which constitutively active AhR may contribute to B lineage cancers is unknown, several possibilities exist. With regard to Burkitt's lymphoma, the AhR complex directly interacts with EBNA-3, a protein required for EBV-mediated cell transformation and involved in cell growth and survival. This interaction enhances AhR nuclear translocation and reporter gene transactivation [127]. Using histiocytic lymphoma, Burkitt's lymphoma, and NHL cell lines, Vogel et al. demonstrated that AhR hyperactivation with TCDD resulted in a loss of the apoptosis response, likely through the modulation of cyclooxygenase-2 (Cox-2) and Bcl-xL [128]. Both Cox-2 and Bcl-xL are known to also inhibit apoptosis in B chronic lymphocytic leukemias [129]. Furthermore, it was noted that TCDD promoted the development of lymphomas and Cox-2 expression in lymphoma-bearing lymph nodes [128]. With regard to TGF-β, a cytokine that inhibits lymphoma apoptosis, AhR has been shown to both suppress [130, 131] and enhance [121] TGF-β expression in a tissue-specific and/or ligand-specific fashion.

Finally, the role of the AhR in the development of B lineage malignancies may not be restricted to the transforming cell itself but may be a function of AhR-dependent events in either the malignant cell or the tumor microenvironment, specifically in AhR^+ bone marrow stromal cells [4, 5, 40, 132]. For example, a constitutively active AhR increases IL-6 production in head and neck cancers [109] and modulates IL-6 production in bone marrow stromal cells [133]. IL-6, produced by the bone marrow microenvironment, is a critical cytokine in the development and maintenance of multiple myeloma [134].

Conclusions

The use of environmental AhR ligands has enabled immunotoxicologists to probe the immune system in order to identify in which cells and at what stage of their development the AhR is expressed and functional. These studies demonstrated that the AhR is variably expressed during B cell differentiation from the HSC to the antibody-secreting cell stage (Fig. 5). These results imply that the AhR is a critical mediator of B cell development and function and that environmental AhR ligands have the potential to adversely affect B lineage cells at multiple levels. The expression of a functional AhR in bone marrow stromal cells further indicates that the AhR may affect B cell development indirectly by altering the function of bone marrow stromal cells critical to B cell growth and differentiation (Fig. 5). Notably, the expression of the AhR in both HSCs and bone marrow stromal cells suggests that the AhR may have a more global effect on other hematopoietic cell lineages, all of which derive from common HSCs and which require signals provided by bone marrow stromal elements.

These studies also point to a possible role for the AhR in B cell malignancies, the incidence of which has risen considerably since 1975 (http://seer.cancer.gov/statfacts/html/nhl.html) [135]. That is, AhR expression in B cell malignancies or in the bone

marrow microenvironment provides a mechanism through which three large classes of environmental chemicals, dioxins, planar PCBs, and PAHs, could contribute to B cell cancers. Of equal importance is the likelihood that the AhR, in the absence of environmental chemicals but, presumably, in the presence of endogenous ligands, contributes to B lineage cancers by influencing cell growth and/or survival either directly within the malignant cell or indirectly via the tumor microenvironment. If proven, this hypothesis would raise the exciting possibility that the AhR signaling pathway could be targeted for B lineage cancer therapy. Thus, studies exploiting environmental chemicals as biologic probes have not only helped to reveal the biological functions of what had previously been thought of only as an environmental chemical sensor but also now suggest a novel strategy for targeted cancer therapy.

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