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Acute Disruption of Bone Marrow Hematopoiesis by Benzo(a)pyrene Is Selectively Reversed by Aryl Hydrocarbon Receptor-Mediated Processes[S]

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ABSTRACT

Bone marrow (BM) hematopoietic cells are selectively sensitive to polycyclic aromatic hydrocarbons (PAH) in vivo. 7,12-Dimethylbenz(*a*)anthracene (DMBA), but not benzo(*a*)pyrene (BP), depletes BM hematopoietic cells in C57BL/6 mice. This difference is due to a BP-selective aryl hydrocarbon receptor (AhR)-mediated recovery. Colony-forming unit assays show suppression of lymphoid progenitors by each PAH within 6 h but a subsequent recovery, exclusively after BP treatment. Suppression of myeloid progenitors (6 h) occurs only for DMBA. Each progenitor responded equally to DMBA and BP in congenic mice expressing the PAH-resistant AhR (AhR^d). AhR, therefore, mediates this BP recovery in each progenitor type. These PAH suppressions depend on Cyp1b1-mediated metabolism. Paradoxically, few genes responded to DMBA, whereas 12 times more responded to BP. Progenitor suppression by DMBA, therefore, occurs with minimal effects on the general BM population. Standard AhR-mediated stimulations (Cyp1a1, Cyp1b1, Ahrr) were similar for each PAH and for the specific agonist 2,3,7,8-tetrachlorodibenzo-*p*-dioxin but were absent in AhR^d mice. A group of 12 such AhR responses was sustained from 6 to 24 h. A second, larger set of BP responses (chemokines, cytokines, cyclooxygenase 2) differed in two respects; DMBA responses were low and BP responses declined extensively from 6 to 24 h. A third cluster exhibited BP-induced increases in protective genes (*Nqo1*, *GST-mu*) that appeared only after 12 h. Conversion of BP to quinones contributes oxidative signaling not seen with DMBA. We propose that genes in this second cluster, which share oxidative signaling and AhR activation, provide the AhR-dependent protection of hematopoietic progenitors seen for BP.

Introduction

Benzo(*a*)pyrene (BP) and 7,12-dimethylbenz(*a*)anthracene (DMBA), two prototypic polycyclic aromatic hydrocarbons (PAHs), impair cell-mediated and humoral immune responses (Ward et al., 1984). PAHs typically require activation by cytochrome P450 to exert their biological effects. PAH-mediated induction of cytochrome P450 transcription is tissue-specific, dependent on aryl hydrocarbon receptor (AhR) activation (Shimada et al., 2003; Galván et al., 2006; Uno et al., 2006). Previous studies showed that intraperitoneal treatment of C57BL/6 mice with DMBA, but not BP, decreases bone marrow (BM) lymphoid and myeloid cellularity through metabolism by Cyp1b1 (Heidel et al., 2000; Galván et al., 2006). This study shows that DMBA and BP can nevertheless produce similar rapid and selective sup-

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ABBREVIATIONS: BP, benzo(a)pyrene; Abc, ATP-binding cassette; AhR, aryl hydrocarbon receptor; Ahrr, aryl hydrocarbon receptor repressor; $B220^+$, 220-kDa variant of CD45, a B-lymphocyte marker; BM, bone marrow; CCL, chemokine C-C motif ligand; CFU, colony-forming unit; Cxcl, chemokine (C-X-C motif) ligand; DBP, dibenzo(*a*,*l*)pyrene; DMBA, 7,12-dimethylbenz(a)anthracene; FBS, fetal bovine serum; GM, granulocyte macrophage; $Gr\text{-}1^+$, granulocyte differentiation marker 1; GST-mu, glutathione transferase mu; HPLC, high-performance liquid chromatography; HSC, hematopoietic stem cells; IL, interleukin; NFκB, nuclear factor κB; Nqo1, NADPH dehydrogenase quinone 1; Nrf2, nuclear factor (erythroid-derived 2)-like 2; p53, tumor suppressor protein; PAH, polycyclic aromatic hydrocarbon; PCR, polymerase chain reaction; PKR, protein kinase R; QPCR, quantitative polymerase chain reaction; Sca-1, stem cell antigen 1; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TNF, tumor necrosis factor; WT, wild type.

pression of lymphoid and myeloid progenitor cells in BM. These PAHs differ because BP selectively directs an AhR-mediated recovery process within hours of administration.

BM hematopoietic cells play central roles in immune responses, angiogenesis, and inflammation (Muguruma et al., 2006; Nagasawa, 2007). The hematopoietic stem cells (HSCs) differentiate into multiple lineages (Singh et al., 2009b). The earliest multipotential progenitor cells (Sca-1$^+$/c-Kit$^+$) differentiate into committed lymphoid progenitors (Sca-1$^+$/c-Kit$^+$ low) and myeloid progenitors (Sca-1$^-$/c-Kit$^+$) cells, which proliferate and differentiate to lineage-specific progenitors that are committed to forming either B-lineage lymphocytes (B220$^+$) or granulocytes (Gr-1$^+$). Hematopoiesis can be recapitulated in vitro by using colony-forming unit (CFU) assays that specifically provide for the expansion of each progenitor type. Here, we use CFU–pre-B and CFU-GM assays, which measure proliferation and differentiation of the respective late progenitors (Igarashi et al., 2006; Nagasawa, 2007). The differentiation of BM cells from HSC can be impaired by various chemical stressors, including PAHs (Allan et al., 2003; Galván et al., 2006; Singh et al., 2009b). These effects may occur either directly on these late progenitors or can affect multiple lineages by targeting earlier progenitors, even HSC. BM contains a high proportion of immature cells, although progenitors remain a small proportion of the total. Immature B lineage BM cells are suppressed to a greater extent by DMBA than mature B cells (Galván et al., 2006).

We have reported previously that DMBA suppresses CFU activities for BM lymphoid (CFU–pre-B) and myeloid (CFU-GM) progenitor cells within 6 h. Myeloid progenitors recover more rapidly after metabolic removal of the DMBA (N′jai et al., 2010). The adverse effects mediated by PAHs are typically the result of metabolism to reactive dihydrodiol epoxides and quinones. Cyp1b1 is a major contributor to this activation, particularly in BM (Heidel et al., 2000; Shimada et al., 2003). Progenitor activities are supported by factors released from adjacent BM stromal cells, including osteoblasts and endothelia. The PAH effects on the BM may result from direct effects of reactive metabolites on progenitor cells and indirect effects of inhibitory factors released from BM stromal cells (Allan et al., 2003). Accordingly, TNFα and other stress response factors (p53 and PKR) are each required for DMBA-mediated disruption of hematopoiesis in vivo (Mann et al., 2001; Page et al., 2004).

AhR activation by PAHs is similar to that caused by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and polychlorinated biphenyls. AhR represses early BM progenitor differentiation, affecting a switch from lymphoid to myeloid lineages (Sakai et al., 2003; Singh et al., 2009b; Boitano et al., 2010). TCDD effects on BM lymphoid progenitors (CFU–pre-B) occur later than we find for DMBA (Singh et al., 2009a; N′jai et al., 2010). The crucial difference is that DMBA suppression is mediated by Cyp1b1 metabolism (Heidel et al., 2000; Galván et al., 2006). Here, we show that BP and DMBA are similarly effective in disrupting BM progenitors within 6 h but differ with respect to a recovery process, exclusive to BP. We use PAH-resistant AhR^d mice to show that BP selectively enhances this recovery through AhR activation. In these studies, we use intraperitoneal administration, which yields a slow release of PAHs from lipid stores that is, in contrast to oral administration (Uno et al., 2004), largely unaffected by induction of Cyp1a1 in the liver (Galván et al., 2003).

Gene expression changes produced by DMBA and BP have been compared from 6 to 24 h after PAH treatment, a period when total cell losses were minimal. Because stromal progenitor cell interactions regulate hematopoiesis (Igarashi et al., 2006; Nagasawa, 2007), BM cells were resolved ex vivo through their adherence to stromal cells. DMBA produces surprisingly few gene responses, whereas BP produces many expression changes, including three sets of responses that differ substantially in their respective time courses. We develop a model for the inter-relationship of these different groups of gene responses in relation to progenitor disruption and recovery.

Materials and Methods

Reagents and Antibodies. DMBA and BP were purchased from AccuStandard, Inc (New Haven, CT) and dissolved in olive oil at a concentration of 5 mg/ml. RPMI 1640 medium was purchased from Sigma Chemical (St. Louis, MO) and was supplemented with 5% (v/v) fetal bovine serum (FBS; Atlanta Biologicals, Norcross, GA), 50 IU/ml penicillin, and 50 μg/ml (w/v) streptomycin. CD45/B220-phycoerythrin and Gr-1-fluorescein isothiocyanate monoclonal antibodies were purchased from BD Pharmingen (Franklin Lakes, NJ). MethoCult methylcellulose media for clonogenic assays were purchased from StemCell Technologies, Inc (Vancouver, BC, Canada). The CFU–Pre-B media (M3630) contained FBS, 2-mercaptoethanol, L-glutamine, and recombinant human interleukin (IL)-7, and the CFU-GM media (M3534) contained FBS, bovine serum albumin, recombinant human insulin, human transferrin, 2-mercaptoethanol, L-glutamine, recombinant mouse stem cell factor, recombinant mouse IL-3, and recombinant human IL-6.

Animals and Treatments. C57BL/6j (wild type, AhR^b) and C57BL/6.D2N-AhR^d (AhR^d) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Cyp1b1(−/−) mice (C57BL/6 background) were bred in our animal care facility, as described previously (Buters et al., 1999). Animals were housed at the Association for Assessment and Accreditation of Laboratory Animal Care-certified University of Wisconsin Madison School of Veterinary Medicine Animal Care Unit and used in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996). Groups of female C57BL/6 mice (6 weeks old) were randomly selected and injected with DMBA or BP (10 or 50 mg/kg i.p.) in olive oil (0.2 ml). This dose of DMBA (50 mg/kg) is similar to those used to initiate tumors in rodent carcinogenicity studies (Medina et al., 1974; Buters et al., 1999) and is also consistent or lower than those used in numerous previously published investigations (Heidel et al., 2000; Galván et al., 2003, 2005, 2006; Uno et al., 2004, 2006; Gao et al., 2005, 2007). Time-matched vehicle control animals were injected with an equivalent volume of olive oil. All mice were euthanized with CO_2 asphyxiation at selected time points, in accordance with University of Wisconsin Madison Institutional Animal Care and Use Committee-approved protocol and the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996).

BM Cell isolation. DMBA- or BP-treated and time-matched vehicle control wild-type (WT), AhR^d, and Cyp1b1(−/−) mice were euthanized by CO_2 asphyxiation. To isolate bone marrow cells, the femurs were dissected free of muscle tissue, and the ends of the bones were removed with a surgical blade. Cells were flushed from the femurs with 2 ml of RPMI 1640 culture medium with the use of a syringe equipped with a 25-gauge needle. A 1:50 dilution of cells in 3% acetic acid was used to enumerate cells in a hemocytometer.

Flow Cytometry Staining and Analysis. Freshly isolated cells were suspended at 10^6 cells per 100 μl of media. Aliquots (100 μl) of

cells were maintained on ice for 20 min with 0.5 μg of purified rat anti-mouse CD16/CD32 (Fcγ III/II Receptor) (Mouse BD Fc Block; BD Biosciencess, San Jose, CA) to block Fc receptors. The cells were then incubated with 1 μg of anti-CD45/B220-phycoerythrin or anti-Gr-1 fluorescein isothiocyanate (to detect B lymphocytes and granulocytes, respectively) for 30 min on ice and washed twice with 200 μl of wash buffer (1% PBS). Cells were resuspended in 200 μl of wash buffer and fixed with 2% paraformaldehyde. Fixed cells were stored in the dark at 4°C, acquired, and analyzed within 3 days. One hundred thousand events were acquired for each sample with a FACSCalibur flow cytometer (BD Biosciences). Populations of B lymphocytes and myeloid cells were gated, as described previously (Thurmond and Gasiewicz, 2000). Data were analyzed using FlowJo 6.4 software (Tree Star, Ashland, OR).

Bone Marrow Cell ColonyForming Unit Assays. Freshly isolated bone marrow cells were resuspended at concentrations of 10^5 cells per ml (CFU–pre-B assay) or 8×10^4 cells per ml (CFU-GM assay) in RPMI 1640 tissue culture medium supplemented with FBS and penicillin/streptomycin. CFU assays were performed following protocols developed by manufacturer (StemCell Technologies). In brief, an aliquot (300 μl) of resuspended bone marrow cells was added to 2.2 ml of CFU–pre-B or CFU-GM methylcellulose (MethoCult) media (StemCell Technologies), mixed by vortexing, and allowed to stand for 5 min for bubbles to dissipate. The bone marrow cells suspended in the MethoCult media were then dispensed into duplicate pretested culture dishes (StemCell Technologies) using a syringe and blunt-ended needle. Bone marrow cells were incubated for 7 to 14 days in a humidified incubator at 37°C and 5% CO_2. Myeloid and lymphoid colonies were evaluated and counted using an inverted microscope and gridded scoring dishes.

Detection of DMBA and BP Blood Levels. Blood samples were collected from the tail vein of C57BL/6 mice at 3, 6, and 24 h after BP or DMBA treatment. The whole -blood sample (10 μl) was diluted in 0.2 ml of 0.5 M NaCl and sonicated for 2- to 15-s bursts at 50% power. After sonication, the blood samples were brought to a total volume of 1.0 ml with NaCl. Dibenzo(*a*,*l*)pyrene (DBP; 50 μl, 0.5 μM) was added to each sample as an extraction control. BP or DMBA and metabolites were extracted by the addition of 2 ml of ethyl acetate/acetone (2:1), with extensive vortexing (2 min), followed by centrifugation. The extraction was repeated with the addition of an additional 1 ml of ethyl acetate/acetone, followed by vortexing and centrifugation. The organic phase was collected and dried under nitrogen gas and stored at −80°C. Bone marrow cells were also similarly extracted. The samples were resuspended in a 20 μl of MeOH immediately before reversed-phase HPLC analysis. DMBA or BP and metabolites were separated on a Beckman C18 Ultrasphere column with a gradient of 50 to 100% MeOH over the first 30 min, followed by sustained 100% MeOH for 25 min. The levels of DMBA or BP and metabolites were quantified by UV (254 nm) and fluorescence detection (excitation and emission wavelengths of 273/395 nm, 5,6- and 8,9 + 10,11-dihydrodiols; 273/470 nm, 7-OH DMBA; 273/415 nm, DMBA and DBP). DMBA or BP levels were quantified relative to known quantities of purified standard.

Separation of Bone Marrow Cell Fractions. To further dissect the bone marrow cell responses, eluted bone marrow cells were separated into an adherent fraction and a nonadherent fraction by attachment to plastic culture dishes. The bone marrow cell suspension from each individual mouse was plated to a single well of a six-well plate. The adherent fraction was allowed to adhere to the plastic during 1.5-h incubation at 37°C. The nonadherent cell population was subsequently collected, whereas the adherent fraction was released by the addition of 1 ml of trypsin. The cells were washed three times with the RPMI 1640 isolation media. The rapid adherence of the stromal cells to plastic was reproducible in multiple mice and has been characterized previously (Phinney et al., 1999; Galván et al., 2006). The adherent fraction comprised approximately 10 to 20% stromal cells, the remainder being hematopoietic cells. In contrast, the nonadherent fraction contained approximately 2 to 4% stromal cells, hematopoietic cells accounting for more than 90%. The presence of $B220^+$ and $Gr\text{-}1^+$ cells was confirmed by flow cytometry.

Microarray Analysis. To further examine the extent of the BM cell responses, we completed gene microarray analyses at 6, 12, or 24 h after intraperitoneal administration of BP and DMBA. Total RNA was isolated from the adherent and nonadherent BM cell fractions using RNeasy mini kits, according to the recommended protocols (QIAGEN, Valencia, CA). At least 6×10^6 cells were used for RNA isolation. RNA was quantified (A_{260}) for concentration, and the purity was determined by A_{260}/A_{280} ratio and by visual inspection of 1.0 μg on a denaturing gel electrophoresis.

All microarray experiments were performed with three biological replicates from individual vehicle-, BP-, or DMBA-treated mice. A reference design labeling of each sample was employed with the use of Agilent mouse genome cDNA arrays containing 44,000 features. For reference design, all samples (vehicle control and treated) from adherent fractions were labeled with cy3 dye. In addition, a reference control pool of nine nonadherent cell fractions samples was labeled with cy5 and used as a control sample. Detailed protocols for microarray preparation, cy3/cy5 labeling of the cDNA probe, sample hybridization, washing, and scanning of Agilent arrays can be found at Bradfield Laboratory, University of Wisconsin Madison. Gene names, symbols, and array identifiers are presented in Supplemental Table 1.

Quantitative Real-Time PCR. Quantitative real-time PCR verification of microarray responses was performed as described previously (N′jai et al., 2008). In brief, 1.0 μg of total RNA was reverse transcribed by SuperScript II using an anchored oligo-dT primer, as described by the manufacturer (Promega, Madison, WI). The cDNA (5 μl) was used as a template in a 25 μl of PCR containing 1 μM forward and reverse gene-specific primers and 12.5 μl of ABsolute Blue QPCR SYBR Green ROX Mix (2×; Thermo Fisher Scientific Inc. UK, Loughborough, Leicestershire, UK). Forward and reverse primer sequences and amplicon sizes are listed in Supplementary Table 2. PCR amplification was conducted on an Applied Biosystems PRISM 7300 Sequence Detection System. cDNAs were quantified using a ΔC_t approach and the copy number of each sample was standardized to two housekeeping genes (Actin B and cyclophilin) to control for differences in RNA loading, quality, and cDNA synthesis.

Data Analysis. All cell data were analyzed by analysis of variance using Prism 5 (GraphPad Software, San Diego, CA).

Results

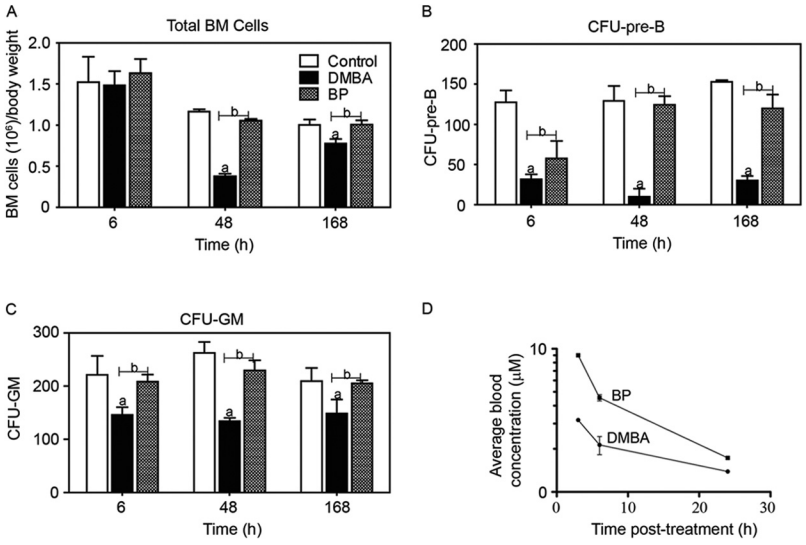
Effects of DMBA and BP on BM Progenitor Cell Populations. CFU assays measure expansion and differentiation of lineage committed BM progenitor cells in semisolid media in response to optimized cytokine mixtures. We compared the effects of intraperitoneal administration of DMBA and BP (50 mg/kg) on lymphoid (CFU–pre-B) and myeloid (CFU-GM) progenitor cells. DMBA and BP each suppressed bone marrow lymphoid (CFU–pre-B) progenitors within 6 h, without any effect on total BM cells (Fig. 1, A and B). DMBA caused a somewhat greater decrease in CFU–pre-B than BP (80 versus 50% decreases) (Fig. 1, A and B). For DMBA, the total cell numbers declined relative to matched controls at 48 h, but recovered appreciably by 168 h (60 versus 25% decreases) (Fig. 1A). There was no effect of BP on BM cellularity. At 48 h, the DMBA suppression of CFU–pre-B declined further, but started to recover by 168 h.

The CFU activities represent colonies derived from a fixed number of plated cells. To compare relative CFU recovered per femur, these CFU activities need to be calculated with respect to changes in total BM cell numbers. Recalculating these data (Fig. 1E) demonstrates that the relative loss of CFU–pre-B at 48 h is greater and the extent of recovery at

168 h is more marked than what is demonstrated by CFU alone. Myeloid progenitor cells (CFU-GM) differed in that suppression by DMBA was consistently less and BP was ineffective, even at 6 h. The attenuated effect on CFU-GM probably arises from the more rapid recovery of myeloid progenitors. This was previously determined for oral DMBA administration, where the PAH is cleared within 12 h (N′jai et al., 2010).

The differences between DMBA and BP did not arise from the blood levels of the chemicals that reach the BM. Although the levels of BP were higher, both followed a similar profile, with high levels at 3 h and a residual component at 6 h, which slowly declined to 24 h (Fig. 1D). We have previously shown, for DMBA, that this decline continues with approximate first-order kinetics out to 168 h, consistent with other work (Uno et al., 2006).

To test the role of AhR in these responses, we used congenic AhR^d mice expressing an Ah receptor, which is resistant to PAH activation. BP and DMBA each produced reductions in CFU–pre-B and CFU-GM progenitor cells in AhR^d mice that are comparable with those seen for DMBA in WT mice (Fig. 2, A–C). These findings suggest that the effects of BP, but not DMBA, are restricted by AhR activation. They further indicate that the ability of BP to diminish CFU–pre-B progenitor activity at 6 h, and its recovery at 48 h, depend on early induction of AhR. Intraperitoneal administration of PAHs provides blood levels at 6 h that are determined by slow release from lipid deposits (N′jai et al., 2010). This

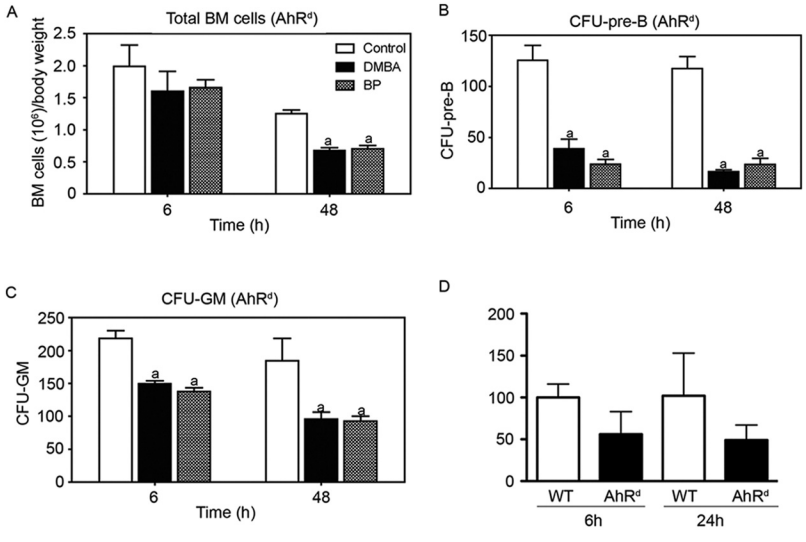


E

CFU/Femur (Oil controls=100)

	Pre-B			GM		
Time (h)	6	48	168	6	48	168
DMBA	25	3	15	70	20	50
BP	40	78	82	100	100	100

Fig. 1. Reduction in BM colony-forming cells (CFU–pre-B and CFU-GM) after intraperitoneal DMBA or BP treatment. Mice received DMBA or BP (50 mg/kg i.p.). Control mice received the same volume (0.2 ml) of oil vehicle. At the indicated times, mice were euthanized and BM cell suspensions prepared as described under *Materials and Methods*. Total BM cells were measured (A) and used to prepare progenitor lymphoid cell (CFU–pre-B) and progenitor myeloid cell (CFU-GM) colony forming assays (B and C). Results are illustrated as the mean ± S.E.M. of four to six animals per group. a, $p < 0.05$ between control and DMBA or BP treatments. b, $p < 0.05$ between DMBA and BP treatment. D, blood was collected via the tail vein, and PAH concentrations were measured by HPLC analysis at 3, 6, and 24 h after treatment. Each time point represents the mean (± S.E.M.) of duplicate animals. E, total CFU per femur was calculated.



E

CFU/Femur (Oil controls=100)

	Pre-B		GM	
Time (h)	6	48	6	48
DMBA	25	5	60	25
BP	15	8	60	25

Fig. 2. BP toxicity is restored in AhR^d mice. AhR^d Mice received DMBA or BP (50 mg/kg i.p.). Control mice received the same volume (0.2 ml) of oil vehicle. At the indicated times, mice were euthanized and BM cell suspensions prepared as described under *Materials and Methods*. Total BM cells were measured (A) and used to prepare progenitor lymphoid cell (CFU–pre-B) and progenitor myeloid cell (CFU-GM) colony forming assays (B and C). Results are illustrated as the mean ± S.E.M. of four to six animals per group. a, $p < 0.05$ between control and DMBA or BP treatments. D, normalized circulating blood levels of BP in WT and AhR^d mice at 6 and 24 h after injection (50 mg/kg i.p.). Each time point represents the mean (± S.E.M.) of three or four different mice. E, total CFU per femur was calculated.

contrasts with oral administration, where first-pass elimination in the liver determines blood levels and is enhanced by AhR induction of Cyp1a1 (Uno et al., 2004). Here, we confirm, in the AhR^d mice used for CFU assays (Fig. 2D), that the switch in AhR genotype does not significantly affect the blood BP level (Galván et al., 2003). In these animals, levels are even somewhat lower and thus cannot account for increased suppression.

Cyp1b1(−/−) Mice Are Resistant to the Effects of DMBA and BP. We next compared the role of Cyp1b1 in the BM response to DMBA and BP. The 6-h CFU–pre-B responses to both DMBA and BP are completely absent in the Cyp1b1(−/−) mice (Fig. 3A). Likewise, the suppression of myeloid progenitor activity (CFU-GM) by DMBA at 6 h is lost in Cyp1b1(−/−) mice (Fig. 3B). It would seem that Cyp1b1 mediates this acute response. This is consistent with our previous finding that suppression of BM lymphoid and myeloid cells by DMBA in WT mice and by BP in AhR^d mice are similarly absent in the Cyp1b1(−/−) genotype. Others have shown that the loss of spleen cellularity is also prevented by Cyp1b1 deletion (Gao et al., 2005), which we have confirmed in our laboratory (data not shown).

We have previously shown that Cyp1b1 deletion does not affect the blood levels of DMBA or DMBA 3,4 dihydrodiol (Halberg et al., 2008) and that BP forms less dihydrodiol epoxide DNA adducts than does DMBA (Galván et al., 2005). Although formation of dihydrodiol epoxide adducts in BM depends on Cyp1b1 (Heidel et al., 2000), the levels of BP dihydrodiols isolated from BM were unaffected by Cyp1b1 deletion (Fig. 3C). These dihydrodiols may therefore arise from other Cyp isoforms, including Cyp1a1, through metabolism in the liver or other sites outside the BM. The ratio of BP to dihydrodiols, however, was much higher in the Cyp1b1(−/−) mice, suggesting that Cyp1b1 metabolism plays a major role in removing BP from BM.

Effects of DMBA and BP on Cell Populations in Bone Marrow. Flow cytometry analyses of bone marrow cells confirm that intraperitoneal DMBA decreases both B cells (B220$^+$) and mature granulocytes (Gr-1$^+$) at 48 h, whereas BP has no effect on numbers of either cell population (Fig. 4A). The B220$^+$ subpopulation remained suppressed 168 h after intraperitoneal administered DMBA, but not BP (Fig. 4B). In contrast, the myeloid Gr-1$^+$ population had recovered appreciably by 168 h after DMBA treatment. In comparison, BP and DMBA produced comparable decreases in the B220$^+$ lymphoid and Gr-1$^+$ myeloid subpopulations in the bone marrow of AhR^d mice (Fig. 4C). We also observed B220$^+$ cell suppression after DMBA, but not BP treatment in the spleen, which is the principal site for maturation of B-lymphoid cells (data not shown). As was noted in the BM, lower B220$^+$ spleen cells were seen in BP-treated AhR^d mice (data not shown).

Selective Basal Expression of Genes in Adherent and Nonadherent BM Cells. Specific stromal-progenitor cell interactions play an important role in hematopoiesis (Muguruma et al., 2006). Ten percent of the total BM cells adhered rapidly to plastic; however, stromal cells represent only a minority of these adherent cells, much as reported previously (Phinney et al., 1999) (Fig. 5A). Hematopoietic cells accounted for almost 90% of the adherent cell population through interaction with the stromal cell surfaces. However, stromal cells are extensively enriched in this adherent fraction relative to the nonadherent fraction (12% versus 2% in nonadherent cells). The proportions of adherent myeloid (Gr-1$^+$) and lymphoid (B220$^+$) cells, determined by flow cytometry, were similar in each fraction and were unaffected by the in vivo PAH treatments (Fig. 5B).

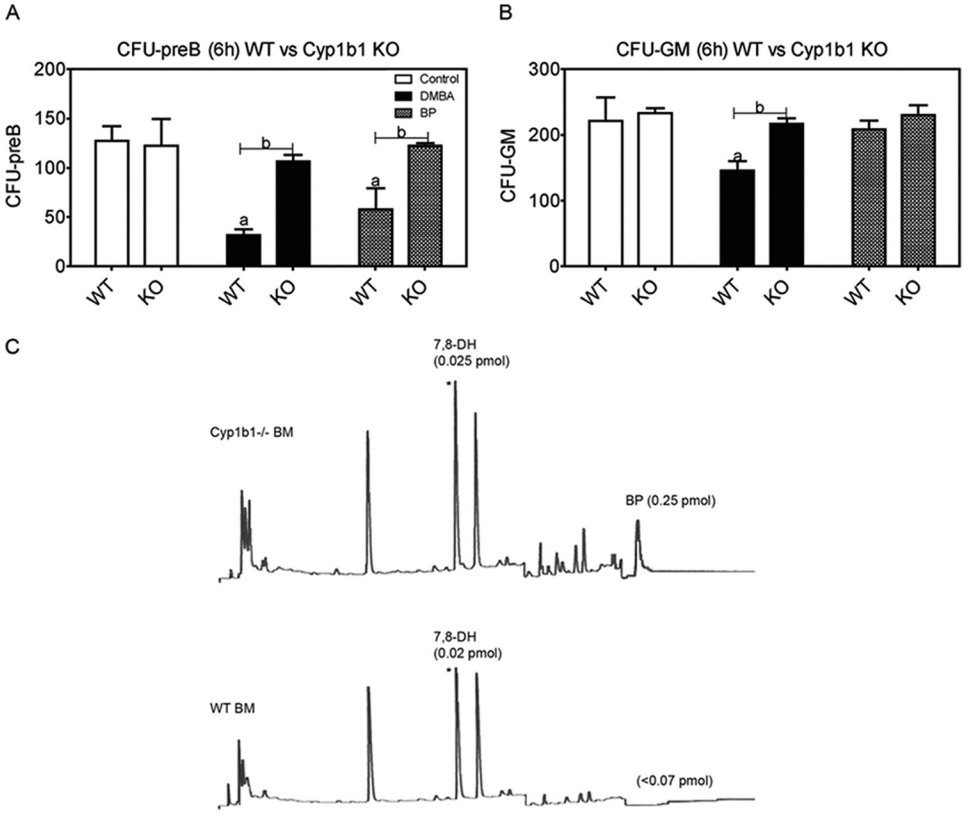


Fig. 3. Cyp1b1(−/−) mice are resistant to the effects of DMBA. Wild-type and Cyp1b1(−/−) mice were injected with DMBA or BP (50 mg/kg i.p.). Control mice received an equal volume of oil vehicle. Results illustrate the mean ± S.E.M. percentage of the response in control mice (three to four mice per group) for BM progenitor lymphoid cells (CFU–pre-B) (A) and BM progenitor myeloid cells (CFU-GM) (B). a, $p < 0.05$ between control and DMBA or BP treatments. b, $p < 0.05$ between DMBA and BP treatment. c, $p < 0.05$ between WT and KO mice. C, Representative HPLC profiles of BP metabolites in the bone marrow of WT and Cyp1b1(−/−) mice 24 h after injection (50 mg/kg i.p.). *7,8-dihydrodiol (7,8-DH) was detectable at equivalent levels in the two strains, whereas BP levels were variable at this time point. Quantification represents PAH and metabolites extracted from BM cells isolated from both femurs of each mouse.

We examined gene expression changes affected by DMBA or BP at 12 h after intraperitoneal administration. This time precedes any decrease in BM cell numbers or shift in major cell populations, as determined by flow cytometry (Fig. 1A). We have focused on the expression changes in the adherent fraction to optimize detection of stromal responses. Surprisingly, there were few DMBA responses (40 genes, fold change >1.6, $p < 0.05$), most of which (33 genes) were shared with BP. By contrast, there were many BP responses (490 genes), most of which were selective (Fig. 5C). For comparison, we

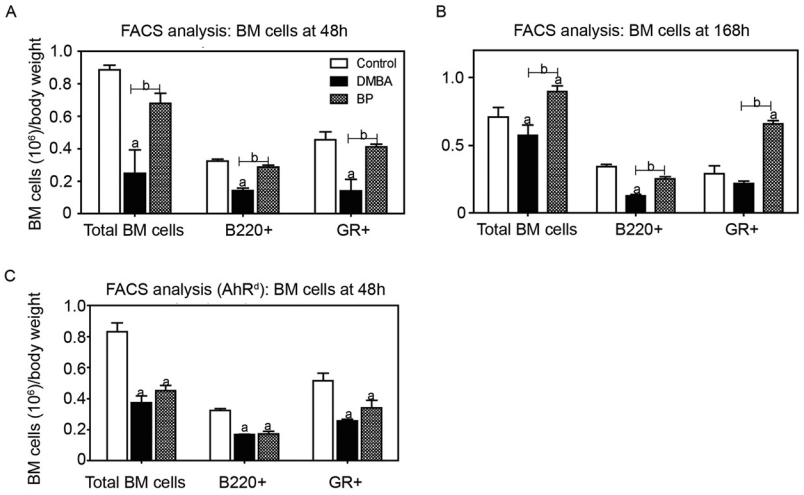


Fig. 4. Decreased BM cellularity in mice after intraperitoneal DMBA, but not BP treatment. Mice received DMBA or BP (50 mg/kg i.p.); control mice received the same volume (0.2 ml) of oil vehicle. At the indicated times, mice were euthanized, and BM cell suspensions were prepared, as described under *Materials and Methods*. These were evaluated for total number of BM cells by hemocytometer and, for B220$^+$ B-lymphocyte lineage cells and Gr-1$^+$ myeloid cells, by flow cytometry (A–C). Total numbers of B220$^+$ and Gr-1$^+$ cells were estimated by multiplying the percentage of BM cells in that population by the total number of BM cells for each mouse. Results are illustrated as the mean ± S.E.M. percentage of the number of cells in vehicle control mice (four to six animals per group); a, $p < 0.05$ between control and DMBA or BP treatments. b, $p < 0.05$ between DMBA and BP treatment.

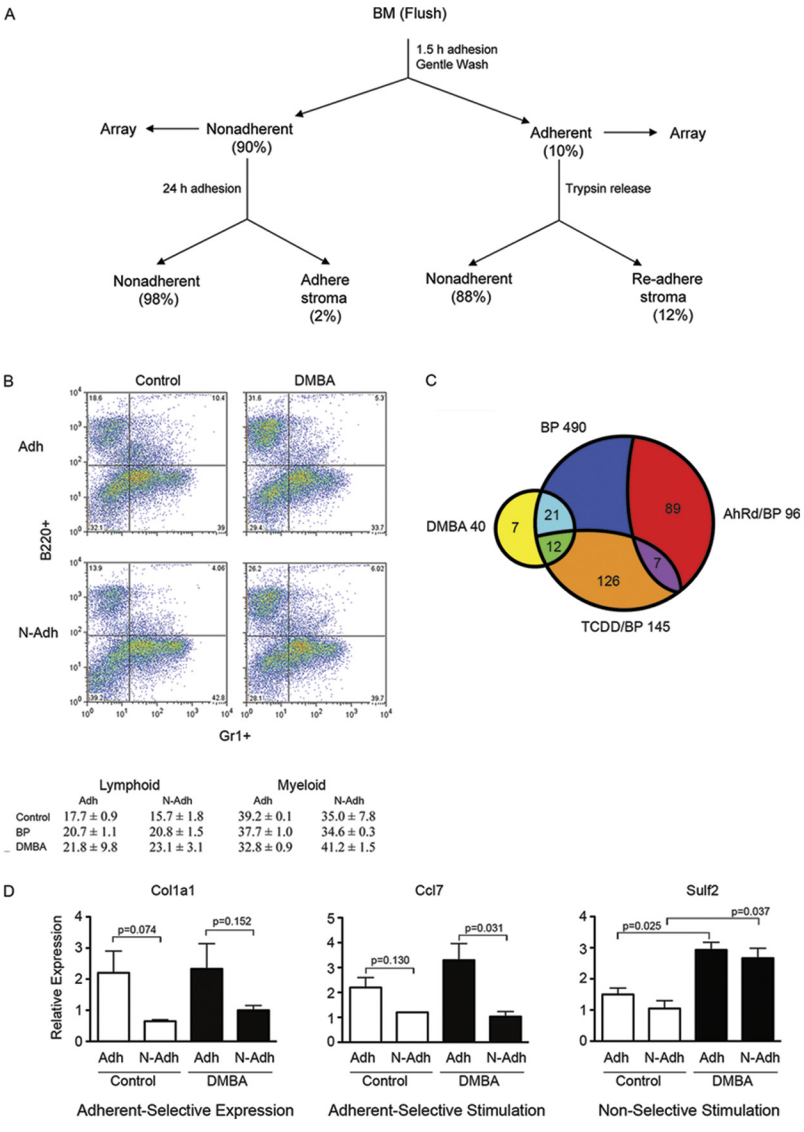


Fig. 5. Separation of BM stromal and hematopoietic cell populations. A, scheme for separation of stromal (adherent) and hematopoietic (nonadherent) bone marrow cell populations. Relative proportions of each population are provided in parentheses. B, flow cytometry analysis of B220$^+$ lymphoid and Gr1$^+$ myeloid populations in the adherent and nonadherent cell populations 24 h after administration of oil, BP, or DMBA (50 mg/kg i.p.), as described under *Materials and Methods*. The scatter plots depict one representative analysis of the separated cells after DMBA treatment. The percentage of cells detected in each treatment/population is summarized in the table. C, selectivity of AhR-mediated gene expression. Microarray analysis was completed in WT or AhR^d mice 12 h after treatment with DMBA (50 mg/kg i.p.), BP (50 mg/kg i.p.), or TCDD (30 μg/kg i.p.). D, Adherence selectivity of gene response.

also evaluated TCDD, which binds potently to the Ah receptor but is not metabolized. Of the 33 responses shared by DMBA and BP, 12 were also shared by TCDD and are therefore probably directly stimulated through AhR activation. BP responses divided into three groups: 30% were reproduced by TCDD, whereas only a further 20% were reproduced in AhR^d mice (lack AhR responses to BP). The remaining 50% were not seen in AhR^d mice (implicating AhR), and were not replicated by TCDD. These responses may result from metabolism of BP to reactive metabolites by either Cyp1a1 or Cyp1b1, each of which is substantially induced by AhR in BM.

Selectivity of Individual Gene Responses. Several genes exhibited 2-fold greater expression in the adherent fraction, with or without DMBA treatment. Collagen 1a1 (Col1a1), a stromal marker (Harrison et al., 2005), typifies this pattern (Fig. 5D; Table 1). For the observed minority of stromal cells in the adherent fraction, this 2-fold selectivity, nevertheless, corresponds to a 15-fold expression selectivity compared with adherent hematopoietic cells. Genes with the reverse selectivity, favoring nonadherent cells, are also apparent (Table 1).

Half of the genes exhibiting DMBA stimulation had increased basal expression in the adherent fraction (Table 2). The chemokine, Ccl7 (Fig. 5D), and two other genes shown in Table 2 were more highly expressed in the adherent fraction and only responded in this fraction. Sulfurase 2 was preferentially expressed in the adherent fraction, but responds similarly in each fraction. We failed to find genes that responded only in the nonadherent fraction. Only six genes followed the selectivity for progenitor disruption and exhibited lower BP responses. These included the cell cycle regulator cyclin G1 and the transcription factor musculoaponeurotic fibrosarcoma oncogene homolog B, a regulator of hematopoietic progenitors.

BP Is Far More Effective than DMBA in Producing BM Gene Responses. Table 3 shows the major BP responses with the corresponding DMBA and TCDD responses. The responses exhibit two extremes: those that show DMBA responses that were similar to BP responses and those that were unaffected by DMBA. A smaller intermediate group shows significant but appreciably lower DMBA responses.

All of these responses are mediated by AhR, because none respond to BP in AhR^d mice. The three classic AhR-linked genes (*Cyp1a1*, *Cyp1b1*, and *Ahrr*) each showed similar responses to BP, DMBA, and TCDD. A further five genes followed these characteristics. We suggest that these are also directly stimulated via AhR. These genes and the five in the intermediate category represent a third of the DMBA re-

TABLE 1
Genes with high selectivity between adherent and non-adherent fractions, but are unaffected by DMBA-treatment *in vivo*

Symbol	Control		DMBA		Cy5
	Adherent	Nonadherent	Adherent	Nonadherent	
Adherent-selective					
Col1a1	2.2	−1.4	2.3	1.0	940
Col1a2	2.1	−1.1	2.0	1.0	860
Col14a1	2.5	1.7	2.0	1.2	240
Cxcl4 (Pf4)	1.6*	−1.1	1.5*	−1.3	7860
Timp3	2.2	1.0	2.1*	−1.3	170
Spic	1.9*	1.0	1.8*	1.0	430
Nonadherent-selective					
Fas1	−2.0	−1.1	−1.7	1.0	290
Polr2a	−1.7	1.4	−2.0	1.5	2060
Ccl5	−2.0*	1.1	−1.7*	1.0	5230

* Significantly different from non-adherent ($P < 0.05$).

TABLE 2
Responses to 12 h of DMBA treatment in vivo

Symbol	Fold PAH Stimulation				
	DMBA		BP Adherent	Control	Cy5
	Adherent	Nonadherent		Adherent/Nonadherent	
DMBA-selective					
Akrlc18	2.2*	2.0	1.2	1.1	250
Ccng1/Cyclin G1	1.9*	1.8	−1.1	1.3	1400
Mafb	1.8*†	1.2	1.0	1.4	1300
Phlda3	1.8*	2.5	−1.3	1.9	500
Thbs1	1.5*	1.2	−1.1	1.1	1360
P2ry5	1.4*	1.2	1.0	1.1	3000
DMBA-nonselective					
Cyp1a1	26.9	19.2	17.0	3.5	10
Cyp1b1	5.3	4.2	5.2	−1.7	30
Ahrr	5.2	6.1	3.8	2.0	60
Hic1	1.6	1.9	5.0	1.2	140
Spint1/Hai	4.3	4.4	4.3	2.0	310
Acpp	2.8	3.1	3.4	−1.1	220
Dpep2	2.8	2.6	2.8	1.3	100
Dnahc2	2.2†	1.4	3.1	1.6	230
Gpnmb	4.1	3.8	4.1	1.3	30
Sulf2	2.1	1.9	1.8	1.4	580
Ctgf	1.8†	1.0	1.6	1.2	300
Ccl7	1.8†	−1.7	1.7	1.8	90
Cdkn1a/p21	2.2	2.5	2.2	−1.3	1550
DMBA-repressed					
Tmem56	−2.0	−2.5	−2.0	1.0	620
Nfia	−2.0	−2.5	−2.0	1.3	440
Aldh1a7	−3.3	−5.0	−5.0	1.3	810
Car1	−2.5	−2.5	−2.5	1.1	6230
Pcna	−1.4	−1.4	−1.7	1.1	67,410

* Significantly greater stimulation by DMBA than BP ($P < 0.05$).
† Significantly greater stimulation in adherent fraction ($P < 0.05$).

sponses and most of the larger increases. The basal expression of genes that exhibited the highest AhR-mediated responses to BP was appreciably higher in the adherent fraction (Table 2). Because the stimulated levels were similar, this high basal expression may represent endogenous AhR activation. This endogenous AhR activation has recently been described for hematopoietic cells in human cord blood (Boitano et al., 2010).

The intermediate category and the most selective BP responses are largely typical of a pro-oxidative inflammatory response: chemokines, cytokines, and cyclooxygenase-2 (Table 2). The BP-selective responses also, for the most part, exhibit TCDD responses that are lower than the BP responses, consistent with an involvement of AhR that is different from the classic response. QPCR analysis confirmed several of the novel AhR responses that shared the profile of Cyp1a1 and Ahrr, the preference for BP compared with DMBA for the inflammatory responses, and the differential expression of selected genes in the adherent cell fraction (Fig. 6).

Time Dependence of BP Responses. Three very different time courses have been identified for these BP responses. Table 4 shows that the eight classic AhR responses changed little between 6 and 24 h. The selective BP responses exhibited a high expression within 6 h, a typical 50% decline at 12 h, but a near-complete disappearance within 24 h. A third category of BP responses was almost the opposite: modest responses in the first 12 h and then a sharp rise at 24 h. These three different time courses are represented in Fig. 7 for Cyp1a1, Cxcl3, and Nqo1.

The delayed responses, summarized in Table 5, show that many derive from genes involved in protection against reactive metabolites (Nqo1, GST-mu, and ATP-dependent Abc-transporters). Two classes of GST (GST-tau and GST-alpha) and several Abc transporters (Abca7, Abca13, Abcb9) were appreciably expressed, but unaffected. Nqo1 responded to BP within 12 h but did not respond to DMBA. Many of the genes in this class have been linked to Nrf2 activation, which has been linked to chemical stress. In the case of Nqo1 and Gst-mu, cooperation with AhR has been reported (Yeager et al., 2009). Other responses follow this pattern. For example, cyclin G1, which is selectively activated by DMBA at 12 h, exhibits this delayed BP response.

TABLE 3
Responses to 12 h of BP treatment in vivo, which are shared with TCDD and separated by DMBA responsiveness

Symbol	BP	BP (AhR^d)	DMBA	TCDD	Cy5
Equal					
Cyp1a1	17.0	−5.0	26.9	14.3	60
Cyp1b1	5.2	−1.1	5.3	1.7	30
Ahrr	3.8	−1.7	5.2	4.8	60
Spint1/Hai-1	4.3	−10.0	4.3	5.2	280
Acpp	3.4	−1.1	2.8	4.3	390
Dnahc2	3.1	−2.0	2.2	2.2	100
Dpep2	2.8	−1.3	2.8	1.5	90
Gpnmb	4.1	−1.7	4.1	1.9	30
Intermediate					
Hic1	5.0	−1.7	1.6	3.8	290
Il1a	7.4	−1.4	3.6	8.7	80
Ptgs2	6.8	1.2	2.4	2.1	220
Ifnb1	10.4	1.2	2.8	8.9	30
Ifrd1	6.5	1.8	2.5	4.3	9410
Cxcl3	7.5	1.2	3.8	5.9	40
Low					
Cxcl2	16.4	1.3	1.8	6.5	200
Il6	1.8	1.1	1.3	3.3	420
Il1b	3.7	1.0	1.6	1.9	10,890
Tnf	3.7	−1.1	1.0	2.3	640
Ccl3	4.3	−1.4	1.3	2.2	6600
Cxcl10	2.2	−1.7	1.3	2.3	1680
Egr1	9.4	−3.3	1.4	1.7	3550
Egr2	2.6	−1.7	1.3	3.7	1490
Nfkbiz	5.0	1.6	1.1	2.9	1740
Tnfaip3	3.8	2.0	1.3	2.7	7160
Socs2	2.0	−10.0	−1.1	1.5	1200
Vpreb1	3.0	1.0	1.0	1.5	17,740
Suppression					
Retnla	−1.3	3.0	−1.1	−5.0	470

Discussion

Several recent studies have implicated the AhR as a constitutive repressor of hematopoietic stem cell differentiation (Singh et al., 2009b; Boitano et al., 2010). Thus, activation by TCDD suppresses hematopoiesis (Singh et al., 2009a). However, we show here that DMBA and BP activate AhR compa-

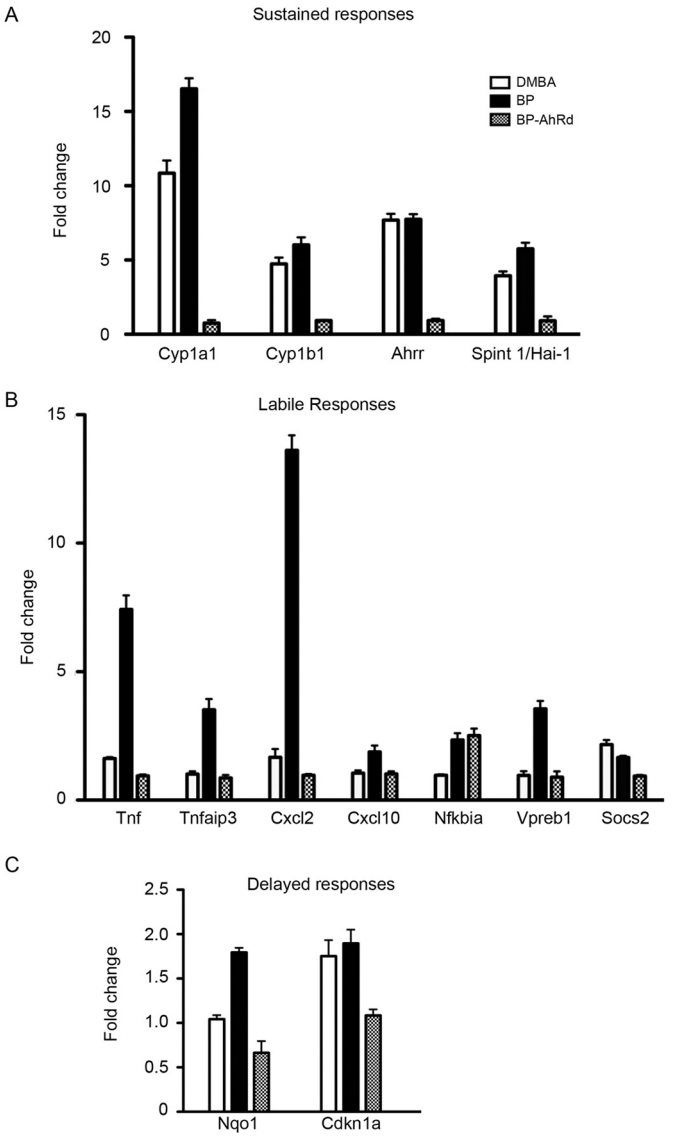


Fig. 6. QPCR of the relative expression levels of selected genes in adherent BM cells after DMBA and BP treatment. Expression levels are shown relative to their expression levels in adherent BM cells from untreated control mice. mRNA isolated from cells from three mice for each treatment were analyzed.

rably with TCDD in BM but disrupt lymphoid and myeloid progenitors more rapidly, despite the need for bioactivation provided by Cyp1b1 metabolism. These two PAHs target lymphoid and myeloid progenitors similarly within 6 h. Paradoxically, BP selectively stimulates an AhR-mediated protection mechanism that also functions remarkably rapidly. These early effects on progenitor activities and their dependence on AhR and Cyp1b1 parallel the later decreases in immature lymphocytes and granulocytes that occur after 24 h (Fig. 3) (Heidel et al., 1998; Galván et al., 2006). The similar disruption effects on myeloid and lymphoid progenitors and their shared AhR-mediated protection each suggest that disruption may actually be directed to their common progenitors. TCDD suppresses the differentiation of such early progenitors (long-term HSCs) and skews differentiation from the myeloid to lymphoid lineage (Singh et al., 2009b). DMBA causes a similar skewing in favor of lymphoid suppression, which may result, however, from faster recovery of myeloid progenitors after PAH disruption (N′jai et al., 2010). This difference may also cause the somewhat lower suppression of myeloid progenitors and their much earlier AhR-mediated recovery.

TABLE 4
Time dependence of BP induction responses

Symbol	6 h	12 h	24 h	Cy5
Sustained responses				
Cyp1a1	20.5	17.0	17.9	10
Cyp1b1	6.4	5.2	8.8	50
Ahrr	10.4	3.8	9.2	110
Hic1	7.6	5.0	5.3	210
Spint1/Hai-1	5.5	4.3	6.6	300
Acpp	4.9	3.4	5.1	680
Dpep2	3.8	2.8	3.1	150
Dnahc2	4.6	3.1	2.7	360
Labile responses				
Cxcl2	20.7	16.4	−3.3	360
Cxcl3	14.6	7.5	2.2	70
Ifnb1	16.5	10.4	1.1	40
Egr1	7.9	9.4	−1.4	4420
Egr2	7.5	2.6	−1.1	1490
Nfkbiz	9.3	5.0	−1.4	2240
Ifrd1	10.6	6.5	2.0	100
Tnfaip3	6.3	3.8	1.4	10,270
Tnf	6.3	3.7	−1.3	1190
Ptgs2	22.4	6.8	1.5	270
Il1a	14.2	7.4	2.1	70
Il1b	6.2	3.7	−3.3	20,010
Il6	4.3	1.8	−3.3	780
Ccl3	4.1	4.3	−1.7	12,410
Ccl7	2.9	1.7	1.7	120
TCDD-independent responses				
Cdkn1a/p21	2.7	2.2	1.9	2040
Ctgf	1.8	1.6	1.2	390

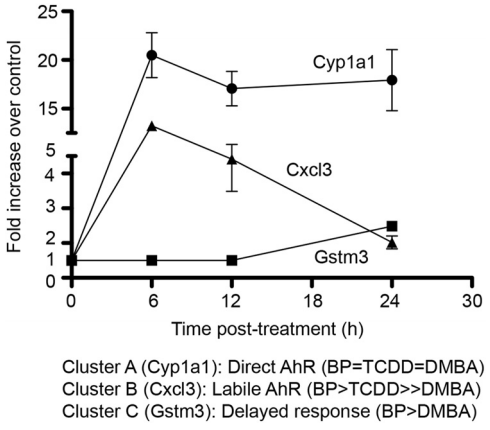


Fig. 7. Cyp1a1, Cxcl3, and Gstm3 are representative of the three classes of AhR-mediated gene response. Microarray analyses were completed 6, 12, and 24 h after PAH treatment.

TABLE 5
Delayed BP induction responses

Symbol	6 h	12 h	24 h	Cy5
Nqo1	1.6	1.6	2.7	1190
Gstm1	1.6	1.1	2.5	1010
Gstm3	1.1	−1.1	3.3	10,480
Gstm4	1.0	−1.1	1.8	200
Abca1	1.3	1.3	2.8	2090
Abcb8	1.0	−1.1	1.8	350
Abcc1	1.5	1.2	1.9	1380
Abcc2	−2.5	−2.0	1.3	50
Abcc5	1.1	1.1	1.7	1460
Abcd2	2.0	1.4	5.6	120
Ptgs1	1.6	1.5	4.1	2150
Ccng1/Cyclin G1	−1.1	1.0	1.9	1660
Sulf2	1.3	1.8	2.9	540

Microarray expression profiling shows surprisingly few DMBA-stimulated gene responses (40 genes) at a time when the CFU activity is extensively suppressed. Only six of these genes were affected more by DMBA than by BP. By contrast, BP produced 490 changes. The DMBA and BP were equally accessible to the BM cells, because typical AhR targets (Cyp1a1, Cyp1b1, and Ahrr) were equally stimulated. This near absence of DMBA responses indicates that most BM cells were completely unaffected when the progenitor cells were suppressed. DMBA may therefore selectively target these early hematopoietic precursors. The abundance of BP responses, including many very large changes in highly expressed genes, suggests that these effects, by contrast, are being realized in predominating cell types. The BP-initiated recovery process may therefore result from intercellular effects on the affected progenitor cells.

It is noteworthy that the PAHs activate AhR to the same extent as TCDD and should be capable of similar effects. The very different outcomes presumably arise because the reactive PAH metabolites appear rapidly enough to redirect the progenitor responses. Cyp1b1, which is expressed selectively within these early common progenitor cells, may increase the effectiveness of disruptive, but labile reactive dihydrodiol epoxides. Equivalent human $CD34^+$ early progenitor cells express appreciable Cyp1b1 (Wang et al., 2005).

Most of the 490 BP responses were lost in AhR^d mice, consistent with mediation by AhR. These BP-stimulated genes included three clusters that exhibited very different time dependence. Classic AhR inductions were similar for DMBA, BP, and TCDD and accounted for 30% of DMBA responses. They retained a constant maximum induction from 6 to 24 h (Table 4). A second set of BP responses was highest at 6 h but declined rapidly. These genes responded to TCDD but only poorly to DMBA (Table 3). This cluster largely comprises genes typically increased in inflammatory responses. The third group of BP responses showed the opposite time course: an increase predominantly between 12 and 24 h (Table 5).

However, several novel AhR targets have been identified that may be relevant to endogenous or TCDD effects of AhR on hematopoiesis. These include the increases in hepatocyte growth factor activation inhibitory protein 1/serine peptidase inhibitor, Kunitz type 1), a notable developmental regulator

(Tanaka et al., 2005). It is noteworthy that appreciable constitutive activation of Cyp1a1, Ahrr, and hepatocyte growth factor activation inhibitory protein 1 was seen in the adherent cells relative to nonadherent cells, possibly replicating the AhR activation seen in human cord-blood hematopoietic progenitors (Boitano et al., 2010).

In the labile response group (Tables 4 and 5), TCDD responses were typically lower than BP responses, especially when DMBA was completely inactive. The functional characteristics of the genes that exhibit AhR-dependent selective responses to BP suggest that oxidative stress or NFκB signaling may mediate the increases. This is suggested by the large increases in cytokines (Tnfα, IL1a, IL1b, and IL6), chemokines (Cxcl2, Cxcl3, Cxcl10), and cyclooxygenase 2 (prostaglandin-endoperoxide synthase 2) along with transcription factors and potential mediators early growth response proteins 1 and 2 (Table 3) (Vogel and Matsumura, 2009).

Many of the delayed response genes (Table 5) are associated with enzymes that provide protection against reactive PAH metabolites. Quinone reductase (Nqo1) reduces BP quinones to hydroquinones (Joseph and Jaiswal, 1998); muglutathione transferases directly conjugate BP dihydrodiol epoxides (Soni et al., 1998); and ATP-binding cassette transporters expel PAH derivatives across the plasma membrane (Lampen et al., 2004). It is noteworthy that many of these genes are induced by AhR in combination with the Nrf2/antioxidant response mechanism (Yeager et al., 2009). This process may account for our previous finding that BP dihydrodiol epoxide adducts form much less readily in BM than the equivalent DMBA adducts (Galván et al., 2005). However, this response is too slow to account for the AhR-mediated protection, which is effective within 6 h.

Oxidative stress is likely to be much more extensive for BP than for DMBA because of the much greater formation of BP quinones. This conversion is extensive for Cyp1a1 but not for Cyp1b1 (Keller and Jefcoate, 1983). These quinones generate oxygen radicals through redox cycling (Joseph and Jaiswal, 1998). Cyp1b1 mediates changes in DMBA dihydrodiol epoxide in BM without appreciable changes in circulating levels (Galván et al., 2005; Halberg et al., 2008) (Fig. 3).

The Cyp1b1-mediated effects on these BM progenitor populations and the AhR-mediated protection from BP disruption are replicated in lymphoid populations in the spleen. These cells substantially derive from progenitor cells, which migrate from the BM (A. U. N′jai, M. C. Larsen, C. R. Jefcoate, and C. J. Czuprynski, manuscript in preparation). This expands on previous studies that have implicated Cyp1b1 and epoxide hydrolase in the effects of PAHs in the spleen (Gao et al., 2005, 2007).

TNF receptor signaling, PKR, and p53-associated stress responses, are also essential participants in DMBA-mediated loss of bone marrow cellularity (Page et al., 2004). Other studies have also shown that ataxia telangiectasia mutated and NFκB activations are involved in the PAH-mediated suppression of spleen cellularity (Gao et al., 2008). Recent work indicates that AhR complexes with NF-κB nuclear factors reticuloendotheliosis viral (v-rel) oncogene related A and B with diverse effects (Vogel and Matsumura, 2009). Synergy and inhibition can arise from the cross talk between TNFα and AhR, probably depending on the cell types involved (Haarmann-Stemmann et al., 2009).

Overall, this work establishes a rapid, acute in vivo effect of PAHs on BM lymphoid and myeloid progenitor cells that is mediated by Cyp1b1 (Fig. 8). The disruptive effects of DMBA metabolism on these progenitors, however, are independent of AhR activation, suggesting activation by low constitutive levels of Cyp1b1 in BM. The selectivity and rapidity of the DMBA-induced changes suggests that Cyp1b1 is more highly expressed in the targeted (Sca-1$^+$) progenitor cells. We showed previously that these increase after DMBA treatment (Galván et al., 2006). The equivalent disruptive effects of BP are limited by AhR activation of a reversal process. The parallel stimulation of genes typically elevated by oxidative stress and NF-κB suggests their participation in the AhR-mediated recovery from BP disruption effects and that formation of quinones distinguishes BP from DMBA. The likely participation of BP-quinones in the AhR-dependent recovery from BP has important implications for risk analysis of PAHs. The many different environmental PAHs differ extensively in the Cyp1b1-mediated production of dihydrodiol epoxides, the activation of AhR and the formation quinones (Shimada et al., 2003).

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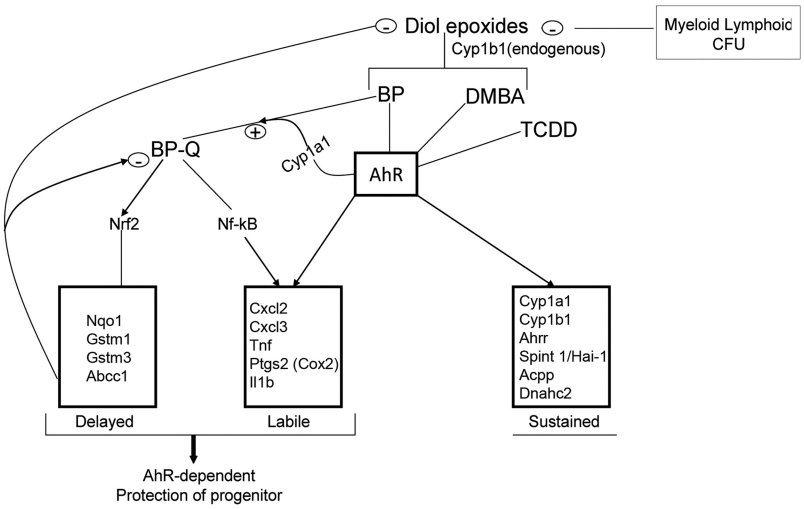

Fig. 8. A scheme to explain the AhR-dependent protection of BM that is selectively directed by BP. PAHs are metabolized by basal BM Cyp1b1 to reactive dihydrodiol epoxides that then damage myeloid and lymphoid progenitor cells. Ensuing gene expression changes suppress responses to stromal signals in vivo or to the CFU stimuli in vitro. More heavily damaged cells may be lost through apoptosis. CFU activity recovers either through replacement of impaired progenitor cells, by stem cell differentiation (HSC), or through facilitation of progenitor cell recovery. AhR activation by BP leads to large increases in inflammatory factors (Table 3). These increases are typical of genes activated by NF-κB, a general inhibitor of apoptosis (Mann et al., 2001). Inhibition of apoptosis may increase the proportion of impaired progenitor cells that can then respond to recovery processes. BP, but not DMBA, is metabolized to BP quinones, primarily by Cyp1a1, which appears only after induction by AhR. Reductive cycling of BP quinones generates reactive oxygen species that stimulate NFκB and many other signaling processes. Other signaling processes stimulate the observed increases in many developmental factors, some of which enhance lineage progression from HSC.

arappa Nanjappa and Dr. M. Suresh for flow cytometry analysis of total bone marrow preparations.

Authorship Contributions

Participated in research design: N′jai, Larsen, Czuprynski, and Jefcoate.

Conducted experiments: N′jai, Larsen, and Czuprynski.

Contributed new reagents or analytic tools: N′jai, Czuprynski, and Jefcoate.

Performed data analysis: N′jai, Jefcoate, Bushkofsky, and Larsen.

Wrote or contributed to the writing of the manuscript: N′jai, Larsen, Czuprynski, and Jefcoate.

Other: Larsen and Bushkofsky bred Cyp1b1 KO mice; Czuprynski and Jefcoate acquired funding for research.

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