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COMPARATIVE ASSESSMENT OF METABOLIC ENZYME LEVELS IN MACROPHAGE POPULATIONS OF THE F344 RAT

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Abstract—The immune system is a direct target for toxic insult by a number of drugs and other chemicals, many of which require activation to toxic metabolites by drug-metabolizing enzymes. We compared the induction of drug-metabolizing enzymes, including cytochrome P450 1A1 (CYP1A1) and aldehyde dehydrogenase (ALDH), which are differentially expressed in various macrophage populations following treatment of F344 rats with the inducer 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Kupffer cells, alveolar macrophages and splenic macrophages from TCDD-treated animals expressed elevated levels of inducible CYP1A1 as compared to other macrophage subpopulations or cells from control rats. TCDD treatment also resulted in increased ethoxyresorufin-*O*-deethylase (EROD) activity and total cytochrome P450 content in tissue-derived macrophages. Immunoreactive protein and mRNA transcripts for CYP1A1 were not detectable in resident peritoneal macrophages or peripheral blood monocytes. Examination of aromatic hydrocarbon receptor (AhR) levels in macrophage populations suggests that the ability of TCDD to induce metabolic enzymes in specific cell types correlates well with AhR expression. *In vivo* activation of macrophages, using either Bacillus of Calmette and Guérin, *Mycobacterium tuberculosis* (BCG) or polyinosinic:polycytidylic acid (Poly I:C), caused no significant alteration in the levels of induction of CYP1A1. ALDH-3 induction was similar in all macrophage populations examined. These studies indicate that macrophages, particularly those from portals of entry, may be induced to produce increased levels of specific enzymes, and the induction is dependent upon their maturational stage rather than their activation state. The metabolism of xenobiotics to toxic intermediates by immune cells and its role in immunosuppression are discussed.

Key words: Cytochrome P450 1A1; macrophages; TCDD; Aldehyde dehydrogenase; Ah Receptor; Drug-metabolizing enzymes

The immune system is a direct target for toxic insult by a number of chemicals and drugs [reviewed in Refs. 1 and 2]. Many of these compounds require metabolic activation in order for their toxicity to be manifested. Although not significantly metabolized, TCDD§, a prototypical immunotoxic chemical, is a potent immunosuppressant in experimental animals, and the immune system appears to be one of the most sensitive target/organ systems to TCDD exposure [3–5]. There is still considerable debate as to the specific mechanism of TCDD-induced immunosuppression *in vivo*, as studies designed to elucidate the mechanism of action have shown that multiple cell types are targeted. Several laboratories have shown that B lymphocytes from mice treated with TCDD or exposed *in vitro* to TCDD have reduced antibody responses [6, 7]. *In vivo* studies suggest that cytotoxic and regulatory T cell function may also be suppressed following TCDD exposure [8, 9]. While it appears that treatment with TCDD does not alter macrophage-mediated antigen presentation, phagocytosis or tumor cell cytolysis and cytostasis, there is evidence suggesting that TCDD treatment may stimulate inflammatory mediators such as reactive oxygen species and inflammatory cytokines [10–12].

Many of the biochemical and toxic effects of TCDD appear to be mediated via binding to the AhR, and suppression of several immune parameters, including T-dependent and -independent antibody responses and cytotoxic T lymphocyte tumor killing are AhR dependent, although there are AhR-independent events as well [reviewed in Ref. 13]. Upon ligand binding, the AhR is activated, and the resultant ligand-receptor complex translocates to the nucleus where it interacts with DREs in the DNA, inducing the transcription of multiple genes, the most studied being a subset of structural genes including those encoding for CYP1A1 [14, 15]. In addition to regulation of CYP1A1, AhR binding to DREs influences the expression of multiple enzymes including CYP1A2, quinone reductase, UDP glucuronosyltransferase, glutathione transferase, and ALDH [16]. In the rat, exposure to a variety of AhR ligands, including PAHs, polybrominated biphenyls, and aromatic amines, results in a receptor-dependent increase in transcription of the ALDH-3 gene in a number of tissues, although the kinetics and dose-dependency are different from those of CYP1A1 induction [17]. This increased transcription results in the production of a class 3 cytosolic ALDH, which participates in the metabolism of aromatic aldehydes, such as those produced as intermediates in primary monooxygenation reactions involving PAHs [17].

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§ Abbreviations: AHH, aryl hydrocarbon hydroxylase; AhR, aromatic hydrocarbon receptor; ALDH, aldehyde dehydrogenase; B(a)P, benzo(*a*)pyrene; BCG, Bacillus of Calmette and Guérin; CYP1A1, cytochrome P450 1A1; dNTP, deoxyribonucleotide triphosphate; DRE, dioxin response element; EROD, ethoxyresorufin-*O*-deethylase; G3PDH, glyceraldehyde 3′-phosphate dehydrogenase; HBSS, Hank's balanced salt solution; MEM, minimal essential medium; PAH, polycyclic aromatic hydrocarbon; PCR, polymerase chain reaction; poly I:C, polyinosinic:polycytidylic acid; RT, reverse transcriptase; and TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

Various forms of cytochrome P450 and/or ALDH activity have been demonstrated in peripheral blood leukocytes and tissue macrophages, as well as in lymphoid cell lines [18–22]. However, there has been little examination of enzyme inducibility in immune cells using purified leukocyte cell populations and/or in various stages of maturation. Immune cells generally demonstrate only a slight capacity to metabolize xenobiotics to reactive intermediates *in vitro*, frequently requiring the use of extracellular enzyme systems derived from tissue homogenates to produce reactive metabolites for *in vitro* model systems [23–25]. The present investigation examined the constitutive and inducible levels of CYP1A1 and ALDH-3 in various populations of macrophages from the F344 rat. Examination of the alterations in enzyme production in varying macrophage populations will provide additional information in determining cell-specific responses to TCDD, and may assist in explaining the cell-specific toxicity of particular xenobiotics, via mechanisms such as the production of reactive metabolites or changes in gene expression leading to alterations in cell function.

MATERIALS AND METHODS

Animals and treatment

Female F344 rats were obtained from Charles River Laboratories (Raleigh, NC) at 9 weeks of age and were maintained on a 12-hr light/dark cycle at 20–22°. The animals were provided autoclaved feed (NIH 31) and water *ad lib.* TCDD (99% purity; Cambridge Isotope Laboratories, Cambridge, MA) was dissolved in a small volume of reagent-grade acetone and subsequently diluted in corn oil to a concentration of 100 μg/mL. Animals were administered a single dose of 100 μg/kg TCDD by gavage in a volume of 0.2 mL. Controls received an equal amount of corn oil. For cell fractionation, mRNA preparation and immunohistochemistry studies, animals were killed in accordance with NIEHS-approved guidelines 8 days following exposure, which allowed for maximal expression of both CYP1A1 and ALDH-3 in the liver [17]. To determine the effects of macrophage activation on CYP1A1 expression, macrophages were activated *in vivo* by i.v. injection of either 5 μg poly I:C (Sigma, St. Louis, MO) or 100 μg of BCG (*Mycobacterium tuberculosis;* Calbiochem, La Jolla, CA) 1 day prior to TCDD treatment. For these studies, animals were killed on day 4, a time period that previous studies in our laboratory determined to coincide with maximum activation in the macrophages examined (data not shown). Macrophage activation was characterized by following the disappearance of 5′-nucleotidase activity [26] at various time points using a commercially available diagnostic kit (Sigma).

Macrophage preparation

Alveolar macrophages were isolated by a modification of the procedure of Warheit *et al.* [27]. Briefly, animals were cannulated, lungs were removed, and the lungs were lavaged with 8 × 5 mL washes of warm HBSS. Cells were centrifuged for 10 min at 400 *g*, and the cell pellets were incubated with 16 mM NH_4Cl in 17 mM Tris for 1 min at 4° to lyse any contaminating red blood cells and washed two times in HBSS.

Resident peritoneal exudate cells were obtained by lavaging the peritoneal cavity with 60 mL of cold HBSS. Cells were pelleted and treated as above. Enrichment of the peritoneal macrophage population was achieved by separation using discontinuous gradients of 44.1, 50.8, 54.6 and 66.7% osmolarity-adjusted Percoll [28].

Kupffer cells were isolated by an *in situ* perfusion technique [29]. Animals were cannulated via the hepatic vein and perfused with oxygenated 0.15% Pronase E (Sigma) in HBSS. The liver tissue was minced and digested with 75 mL of the above buffer for 1 hr at 37°. The digesting solution was filtered and diluted, and the cells were pelleted and washed three times in HBSS. Pelleted cells were resuspended and separated by centrifugation in a solution of 50% Percoll. After washing in HBSS, isolated Kupffer cells were allowed to adhere to plastic culture dishes by incubation in MEM (Life Technologies, Gaithersburg, MD) for 30 min at 37°. Nonadherent cells were removed by washing with HBSS, and the adherent Kupffer cells were collected by scraping.

Splenic adherent cells were obtained by aseptically removing the spleen and preparing single cell suspensions in HBSS [7]. Nucleated cells were adjusted to 2×10^6/mL in MEM and allowed to adhere to plastic culture flasks for 2 hr at 37°. Nonadherent cells were removed by washing with HBSS, and the adherent cells were removed by scraping.

Peripheral blood monocytes were isolated from heparinized whole blood. Animals were anesthetized with CO_2, and 5 mL of blood was removed from the right ventricle of the heart. The blood from control or treated animals was pooled, diluted in HBSS (1:5), and layered over Lympholyte R™ (Accurate Chemical, Westbury, NY). The gradients were centrifuged for 20 min at 1500 *g*, and the purified mononuclear cells were collected from the interface, washed three times in HBSS, and allowed to adhere to plastic culture flasks for 2 hr at 37°. Nonadherent cells were removed as described above. The purity of all macrophage populations was confirmed by cytospin preparations stained with Diff-Quik™ solutions (Baxter, Miami, FL), and found to be >90%.

Cell fractionation

Macrophage preparations or 50 mg of liver tissue from individual animals were pooled prior to fractionation. Microsomes were isolated from tissues by homogenization in 50 mM potassium phosphate, 0.1 mM EDTA, 1.15% KCl, pH 7.5, as previously described [30]. The homogenates were centrifuged at 3,000 *g* for 10 min to remove undisrupted cells and large cell fragments, followed by 100,000 *g* for 1 hr to sediment the microsomes. The 100,000 *g* supernatants were concentrated in a speed vacuum and frozen at −80° prior to determining immunoreactive ALDH. The microsomal pellets were resuspended in 50 mM potassium phosphate, 0.1 mM EDTA, 0.25 M sucrose, pH 7.5, and frozen at −80° for later use. Protein concentration of microsomal and cytosolic fractions were determined using bicinchoninic acid protein assay reagent (Pierce, Rockford, IL) with BSA as a standard. For AhR studies, cytosolic fractions were obtained by homogenizing a minimum of 10^8 macrophages in 0.4 mL HEDG buffer (25 mM HEPES, 1.5 mM EDTA, 1 mM dithiothreitol, 10% glycerol, pH 7.6) as previously described [31]. The homogenates were centrifuged at 100,000 *g* for 1 hr at 4°, and the supernates containing the cytosolic fraction were removed and stored at −80° until used for immunoblotting. Protein concentration of the AhR cytosolic

fractions was quantitated using the Bio-Rad Protein Assay Reagent (Hercules, CA) with BSA as a standard.

Immunoblotting and enzyme assays

To quantitate immunoreactive enzymes, 20 μg of cytosolic or microsomal protein from the macrophage preparations or 2 μg of cytosolic or microsomal protein from the whole liver preparations was electrophoresed in 12.5% polyacrylamide gels and electroblotted to nitrocellulose. Blots were probed with a 1:50 dilution of antibody directed against ALDH-3 (supplied by Dr. R. Lindahl, University of South Dakota) or cytochrome P4501A1 (Oxygene, Dallas, TX) and stained with alkaline phosphatase using the substrate 5-bromo-4-chloro-3-indoyl phosphate and nitroblue tetrazolium color reagent (Bio-Rad Laboratories, Richmond, CA). To examine AhR levels, 40 μg of cytosolic preparations in HEDG buffer was electrophoresed on 7.5% SDS–polyacrylamide gels, electroblotted to nitrocellulose, probed with a 4 μg/mL solution of anti-AhR antibody (supplied by Dr. G. Clark, NIEHS) and stained as described above. Quantitation of immunoblots was performed by scanning on an UltroScan Laser Densitometer (Pharmacia-LKB, Piscataway, NJ). EROD activity was assayed by a direct fluorometric method [32] using 200 μg of macrophage microsomes in 1.5 nmol ethoxyresorufin, 0.25 mM NADPH, 0.1 M potassium phosphate, 0.1 mM Mg^{2+}, pH 7.6, and measuring the linear increase in fluorescence concomitant with the conversion of ethoxyresorufin to resorufin. Total cytochrome P450 in the microsomal preparations was determined by diluting 50–200 μg microsomal protein in 0.1 M potassium phosphate, pH 7.6, and reducing with carbon monoxide using the indirect method of Bickers *et al.* [33]. Baseline-corrected spectra were obtained in a split beam spectrophotometer using one cuvette reduced with dithionite. The differences in spectra were recorded, and total P450 was calculated from the formula:

$$\text{Total P450} = \frac{\Delta\text{Abs } 450/490 \cdot \text{AU} \cdot \text{Dilution}}{\text{Extinction coefficient } (0.100)}$$

RNA extraction, reverse transcription and PCR amplification

Cellular poly(A^+) mRNA was isolated by binding to oligo d(T) cellulose spin columns (Invitrogen, San Diego, CA). The RNA was reverse transcribed by incubating samples for 30 min at 42° in a reaction buffer containing 15 mM Tris–HCl, 37.5 mM KCl, 10 mM dithiothreitol, 1.5 mM $MgCl_2$, 10 mM of each dNTP (Perkin-Elmer Cetus, Norwalk, CT), 400 U Superscript reverse transcriptase (Life Technologies) and 0.5 mg oligo d(T) 12–18 primer (Life Technologies). The reaction was stopped by incubation at 99° for 5 min. A 7-μL aliquot of cDNA (corresponding to approximately 10 ng of mRNA) from each sample was amplified for 35 cycles by PCR in 43 μL of a mixture containing 5 μL of 10 × PCR buffer (100 mM Tris–HCl, 500 mM KCl, pH 8.3), 1 mM of each dNTP, 20 mM specific primers, and 0.25 μL DNA polymerase (Perkin-Elmer Cetus). A rat CYP1A1-specific primer targeted to sites in the cDNA flanking the intron/exon boundary of the corresponding genomic regions of exons 6–7 and producing a 341 bp product was used to examine changes in cytochrome P450 gene expression [34]. The sense primer 5′-CCATGACCAGGAACTATGGG-3′ and anti-sense primer 5′-TCTGGTGAGCATCCAGGACA-3′ were synthesized at the Duke University DNA Synthesis Facility (Durham, NC) and were used in conjunction with the G3PDH (Clontech Laboratories, Palo Alto, CA) sense primer 5′-TGAAGGTCGGAGTCAACGGATTTGGT-3′ and anti-sense primer 5′-CATGTGGGCCATGAGGTCCACCAC-3′ to standardize mRNA concentrations. RNA concentrations and PCR cycles were titrated to established standard curves, to document linearity and to permit semi-quantitative analysis of signal strength. The PCR products were visualized by UV illumination following electrophoresis through a 2% agarose gel and staining in Tris-borate-EDTA buffer containing 0.5 μg/mL ethidium bromide.

RESULTS

Examination of CYP1A1 levels in macrophage populations by immunoblot demonstrated a differential response among the cell types in their ability to produce immunoreactive CYP1A1 after treatment with TCDD (Fig. 1A). Liver microsomal protein was included as a positive control. Densitometric quantitation indicated that the enzyme levels increased 5-fold in alveolar macrophages, 3-fold in splenic adherent cells and >200-fold in Kupffer cells when TCDD-treated animals were compared with controls (Fig. 1B). Immunoreactive CYP1A1 could not be detected in macrophages from control animals or in resident peritoneal macrophages from animals treated with TCDD. In contrast, flavin-containing monooxygenase activity was found exclusively in peritoneal macrophages and not in other macrophage populations (data not shown). All macrophages from TCDD-treated animals demonstrated at least an 8-fold induction in immunoreactive class 3 ALDH enzyme as compared with non-treated controls (Fig. 2A). Since ALDH-3 induction was similar in all populations examined, no further comparisons were made with this enzyme.

To determine whether the CYP1A1 detected by immunoblot was functional enzyme, EROD activity was examined from microsomes prepared using macrophages obtained from control and TCDD-treated animals. Consistent with the levels of immunoreactive enzyme, EROD activity was elevated significantly in alveolar macrophages, Kupffer cells, and splenic adherent cells, and only slightly increased in peritoneal cells after TCDD treatment (Table 1). A comparison of total P450 levels indicated that cytochrome P450 content was increased in alveolar macrophages, splenic adherent cells, and whole liver, while the levels remained constant in peritoneal macrophages from either control or TCDD-treated animals (Table 2).

As P450 levels in alveolar macrophages were increased markedly after TCDD treatment, the kinetics of macrophage CYP1A1 expression was determined and compared with that in the liver. As shown in Fig. 3, immunoreactive CYP1A1 levels rose rapidly in the livers of treated animals, peaking after a 250-fold increase at 48 hr, which was maintained for at least 192 hr. Induction was slightly different in the alveolar cells, with peak levels not achieved until 96 hr after TCDD treatment, though the 60-fold increase was sustained as long as that in the liver.

Fixed tissue macrophages, in which CYP1A1 induction was found to occur, represent a more mature macrophage population than monocytes, such as those that

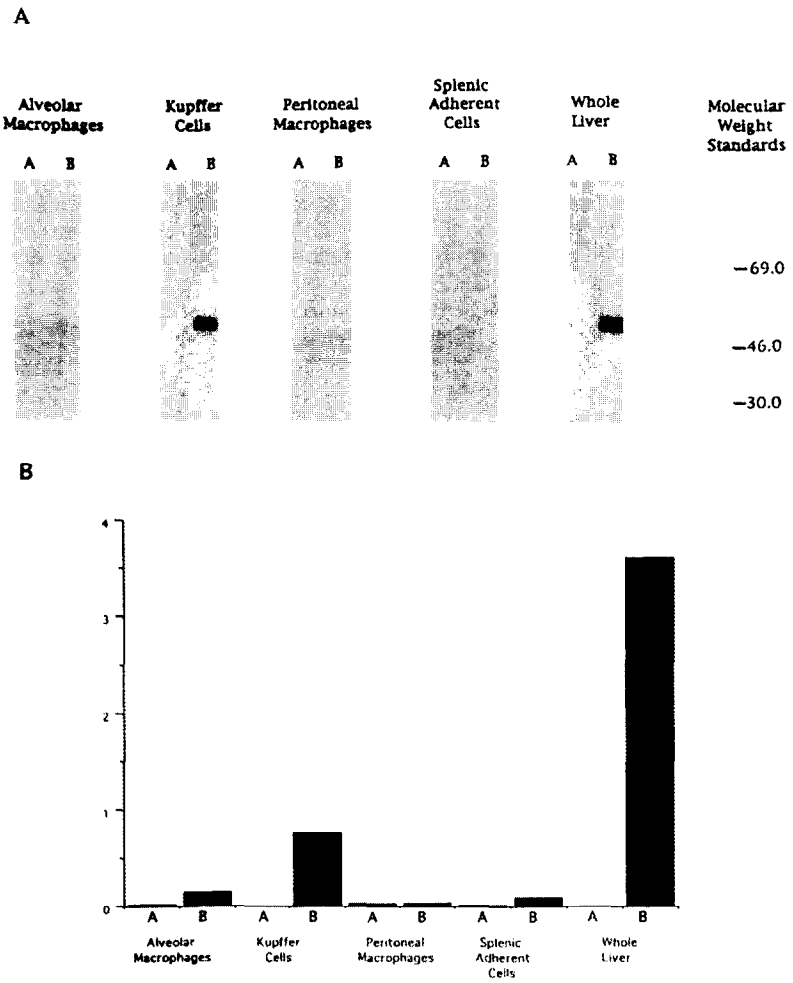

Fig. 1. Comparison of immunoreactive CYP1A1 in macrophage populations. (A) Microsomes from macrophage preparations or matching liver were prepared, electrophoresed, and blotted as described in Materials and Methods. Twenty micrograms of macrophage microsomes or 2 μg liver microsomes was applied to each lane. Column A = corn oil-treated controls. Column B = *in vivo* induction with 100 μg/kg TCDD. (B) Laser densitometry readings from comparative macrophage western blots.

circulate in the blood or reside in the peritoneum. To investigate further the effect of cell maturity on CYP1A1 inducibility, RT-PCR was used to characterize CYP1A1 mRNA expression in macrophage populations, including peripheral blood monocytes. RT-PCR allows for high sensitivity with the use of a limited sample size. PCR analyses confirmed the earlier enzyme results in that induction of CYP1A1 mRNA occurred in both alveolar macrophages and splenic adherent cells after TCDD treatment (Fig. 4). However, neither the peritoneal macrophages nor the peripheral blood monocytes from treated animals expressed any detectable mRNA for CYP1A1.

To determine whether the differential induction of CYP1A1 in macrophages was related to expression of the AhR in these cells, we examined the expression of immunoreactive AhR protein (Fig. 5). Immunoblots demonstrated low levels of AhR in cytosols from freshly isolated alveolar macrophages. However, immunoreactive receptor protein was not detectable in cytosols prepared from peripheral blood monocytes or peritoneal macrophages.

Tissue-fixed macrophages are functionally more mature than are either peripheral blood monocytes or peritoneal cells, the latter requiring stimulation to demonstrate characteristics of early stages of activation such as cytokine secretion and enhanced phagocytosis [35]. To examine whether the activation state influenced the inducibility of macrophage populations, animals were administered either 5 μg poly I:C or 100 μg BCG 1 day prior to TCDD treatment. As shown in Fig. 6, poly I:C or BCG administration did not affect the ability of peritoneal macrophages to form immunoreactive CYP1A1. EROD activity was also unaffected in these cells after *in vivo* activation with BCG (Table 3). Macrophage activation was confirmed by the loss of 5′-nucleotidase activity (38.72± 2.8 U/L in control vs 12.23 ± 0.4 U/L in poly I:C-treated animals and 8.68 ± 0.6 U/L in BCG-treated animals).

DISCUSSION

ALDH induction after treatment with TCDD may be an important mechanism by which cells, including those of the immune system, detoxify reactive intermediates, as this enzyme functions in the metabolism of aromatic

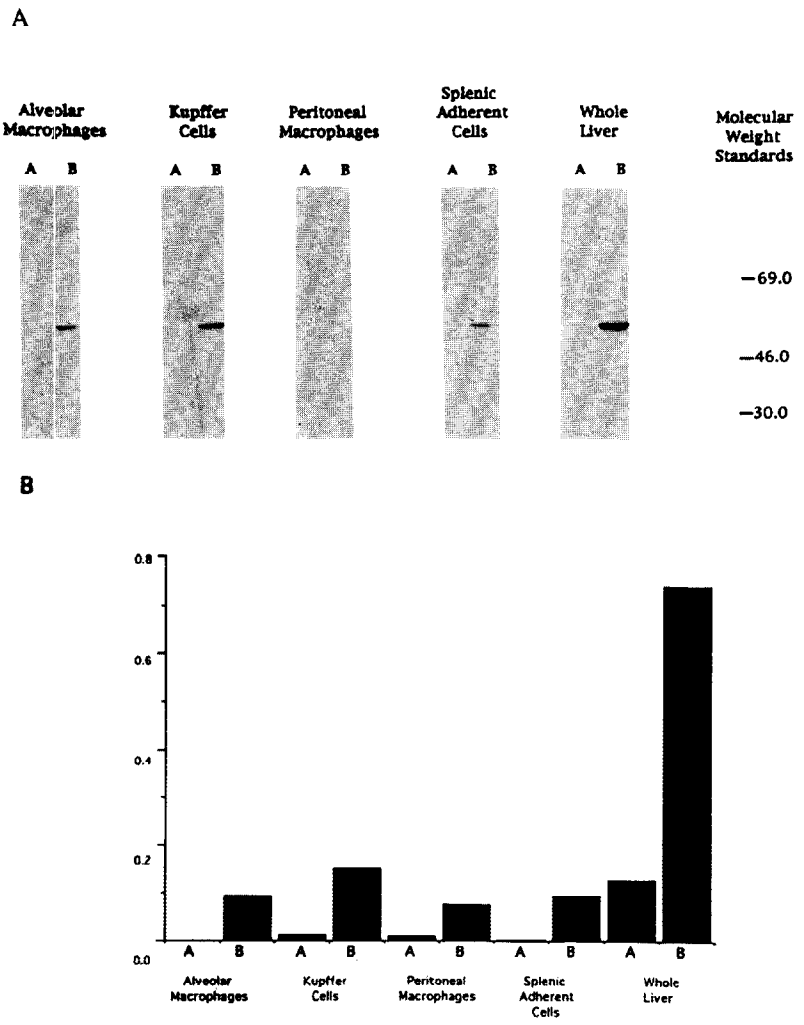

Fig. 2. Comparison of immunoreactive ALDH in macrophage populations. (A) Cytosol from macrophage preparations or matching liver was prepared, electrophoresed, and blotted as described in Materials and Methods. Twenty micrograms of macrophage cytosolic protein or 2 μg liver cytosolic protein was applied to each lane. Column A = corn oil-treated controls. Column B = *in vivo* induction with 100 μg/kg TCDD. (B) Laser densitometry readings from comparative macrophage western blots.

aldehydes, such as those produced in primary monooxygenation reactions involving PAHs [17]. The specific isozyme examined in these studies is one of the three cytosolic ALDHs, ALDH-3, which is induced by a variety of carcinogens including PAHs, polybrominated biphenyls and aromatic amines [36]. The other cytosolic ALDHs are induced by type I compounds, such as phenobarbital. ALDH enzymes reduce the myelotoxicity of cyclophosphamide in bone marrow cells and mediate drug tolerance in resistant tumor cell lines [37, 38]. While specific cell populations have not been examined previously, ALDH-3 has been detected in lymphoid organs. Dunn *et al.* [17] found that TCDD treatment increases expression of class 3 ALDH mRNA in the rat spleen, with no corresponding increase in CYP1A1 message. In contrast, a high increase in CYP1A1 mRNA in the thymus was found, with only a slight increase in class 3 ALDH mRNA expression. This suggested that T lymphocytes are not responsible for the increased ALDH-3 activity and is supported by studies in neonatal chicks that have elevated levels of ALDH in the bursa, but not the thymus after treatment with 3,3′,4,4′-tetrachloro-biphenyl [21]. These studies extend those of Dunn *et al.* [17], which indicate that induction of ALDH-3 gene expression is not highly correlated with the tissue distribution of the AhR, suggesting that its induction in immune cells and tissues may be an AhR-independent non-specific adaptive response to altered cellular physiology or oxidative stress.

The ability to induce drug-metabolizing enzymes in selected macrophage populations, such as Kupffer cells, alveolar macrophages, and splenic adherent cells, was not surprising since many cell types from mesodermal and ectodermal origins have metabolic capabilities [39]. Our studies indicate that Kupffer cells have the highest levels of induction of all macrophage populations examined, and support earlier studies demonstrating increased levels of AHH [18, 40] and EROD [41] activity in these cells. However, it is possible that the reported Kupffer cell activity may be due to cross-contamination from phagocytosis of hepatocyte debris during the isolation procedure. As the liver consistently demonstrates at least a 30-fold higher level of immunoreactive CYP1A1 than either the alveolar or splenic macrophages, a small quan-

Table 1. Ethoxyresorufin-*O*-deethylase activity in macrophage microsomes

Cell population*	EROD activity (pmol/mg/min)
Alveolar macrophages	
Control	2.4 ± 0.4†
TCDD-treated	9.0 ± 0.7‡
Kupffer cells	
Control	1.4 ± 0.5
TCDD-treated	10.1 ± 0.8‡
Peritoneal macrophages	
Control	0.9 ± 0.1
TCDD-treated	1.6 ± 0.2
Splenic adherent cells	
Control	0.7 ± 0.2
TCDD-treated	4.3 ± 0.3‡
Whole liver microsomes	
Control	29.7 ± 1.0
TCDD-treated	2203.1 ± 104‡

* F344 rats were administered a single dose of either corn oil or 100 μg/kg TCDD. Microsomes were isolated from macrophage or tissue preparations after 8 days, and EROD activity was measured by fluorometry and described in the Materials and Methods.

† Mean ± SEM of three replicates.

‡ Significantly different from control at $P < 0.01$ by Student's *t*-test.

tity of contaminating material could skew the activity in Kupffer cells.

Pulmonary alveolar macrophages were found to produce elevated levels of CYP1A1 mRNA and functional enzyme in response to treatment with TCDD. This is consistent with previous studies examining the metabolic capabilities of alveolar macrophages. For example, Tomingas *et al.* [42] report elevated AHH activity in guinea pig alveolar macrophages exposed to B(a)P *in vitro*. Human alveolar macrophages also demonstrate elevated enzyme activity in response to B(a)P [43], and monooxygenase activity has also been shown in lung macrophages from rabbits [44]. Splenic adherent cells in the rat exhibit relatively lower levels of CYP1A1 than do alveolar macrophages, but are clearly inducible after TCDD treatment. Ladics *et al.* [22] have shown that macrophages are responsible for B(a)P metabolism in mouse spleen cells, which in turn modulate immunotoxicity as observed *in vivo* [45]. Splenic macrophages have also been implicated in the cytochrome P450-dependent metabolism of certain drugs, such as chlorpromazine and diphenylhydantoin, which induce localized tissue damage in the spleen [46].

Examination of AhR levels in macrophage populations suggests that the ability of TCDD to induce metabolic enzymes in specific cell types correlates well with AhR expression, and the differences in TCDD responsiveness in the macrophage populations examined are likely related to differential AhR expression. Indirect evidence that TCDD induces AhR-mediated changes in

Table 2. Total cytochrome P450 content in macrophage microsomes

Cell population	Cytochrome P450 content* (nmol/mg)
Alveolar macrophages	
Control	0.28
TCDD-treated	1.09
Peritoneal macrophages	
Control	0.40
TCDD-treated	0.42
Splenic adherent cells	
Control	0.59
TCDD-Treated	1.14
Whole liver microsomes	
Control	11.9
TCDD-treated	29.7

* F344 rats were administered a single dose of either corn oil or 100 μg/kg TCDD. Microsomes were isolated from macrophage or tissue preparations after 8 days, and the total cytochrome P450 content was calculated from indirect difference spectra as described in Materials and Methods using the formula:

Total P450 = ΔAbs 450/490 · AU · Dilution/Extinction coefficient (0.100)

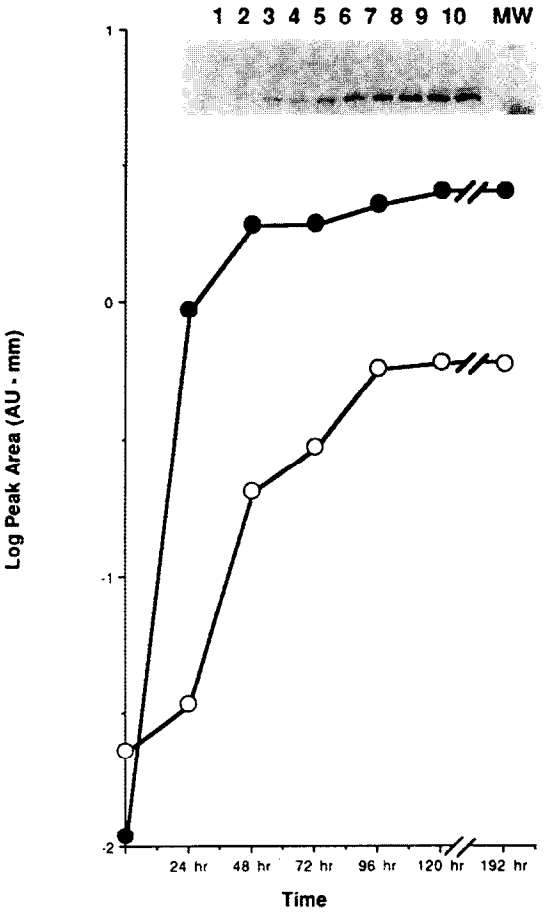

Fig. 3. Kinetics of the expression of immunoreactive CYP1A1 in alveolar macrophages as compared with liver. Microsomes from alveolar macrophages (○) and whole liver (●) from TCDD-treated rats were prepared at selected time intervals as described in Materials and Methods. Twenty micrograms of macrophage and 2 μg of liver microsomes from each time point were electrophoresed as described in Materials and Methods. Inset: 1–5 = alveolar macrophage microsomes; 6–10 = whole liver microsomes collected at 24, 48, 72, 96 and 120 hr post-TCDD treatment. MW = molecular weight markers.

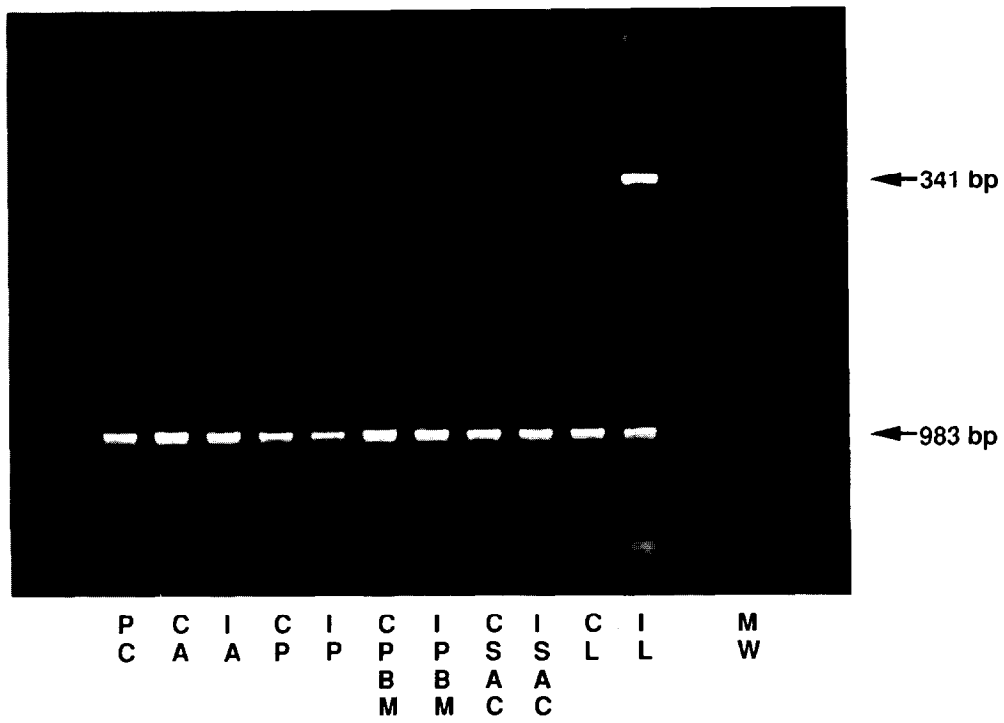

Fig. 4. RT-PCR amplification of CYP1A1 and G3PDH mRNA. cDNA from varying macrophages was prepared using poly(A^+) mRNA. Aliquots (7 μL) of cDNA (corresponding to 10 ng mRNA) were amplified for 35 cycles by PCR using rat CYP1A1 or G3PDH primers. The PCR products were visualized by UV illumination following electrophoresis through a 2.5% agarose gel and staining in Tris-borate-EDTA buffer containing 0.5 μg/mL ethidium bromide. PC = commercial G3PDH positive control cDNA; CA = control alveolar macrophages; IA = TCDD-treated alveolar macrophages; CP = control peritoneal macrophages; IP = TCDD-treated peritoneal macrophages; CPBM = control peripheral blood monocytes; IPBM = TCDD-treated peripheral blood monocytes; CSAC = control splenic adherent cells; ISAC = TCDD-treated splenic adherent cells; CL = control liver; IL = TCDD-treated liver; and MW = molecular weight standards.

macrophage function has been demonstrated in the work of Alsharif *et al.* [47]. These authors demonstrated that TCDD-induced alterations in the production of reactive oxygen species by macrophages *in vitro* were influenced by the genetic background specifying *Ah* responsiveness in the animals from which the cells were isolated.

Our studies suggest that CYP1A1 levels in macrophages are associated with the stage of cell maturation. Relatively immature monocytes, such as those that reside in the peritoneal cavity or peripheral blood, had little or no detectable CYP1A1, whereas more mature tissue macrophages expressed increased levels of con-

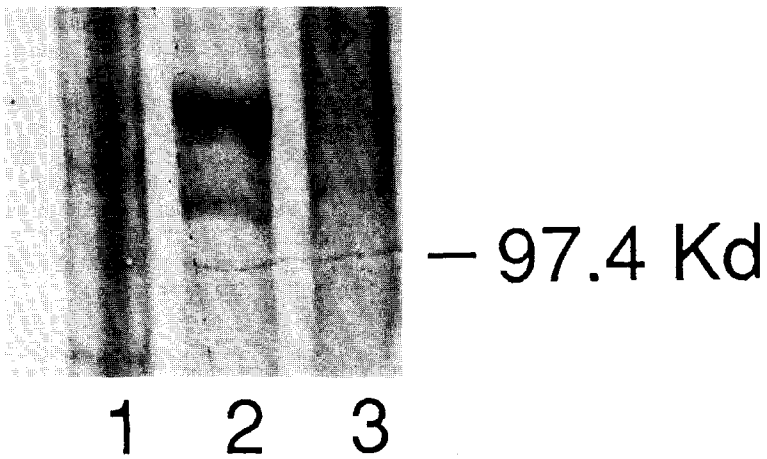

Fig. 5. Cytosolic AhR in macrophage populations. Cytosols from purified macrophages were prepared, electrophoresed, and immunoblotted as described in Materials and Methods. Cytosolic protein (40 μg) was applied to each lane. Lane 1 = peripheral blood monocytes; Lane 2 = alveolar macrophages; and Lane 3 = peritoneal macrophages.

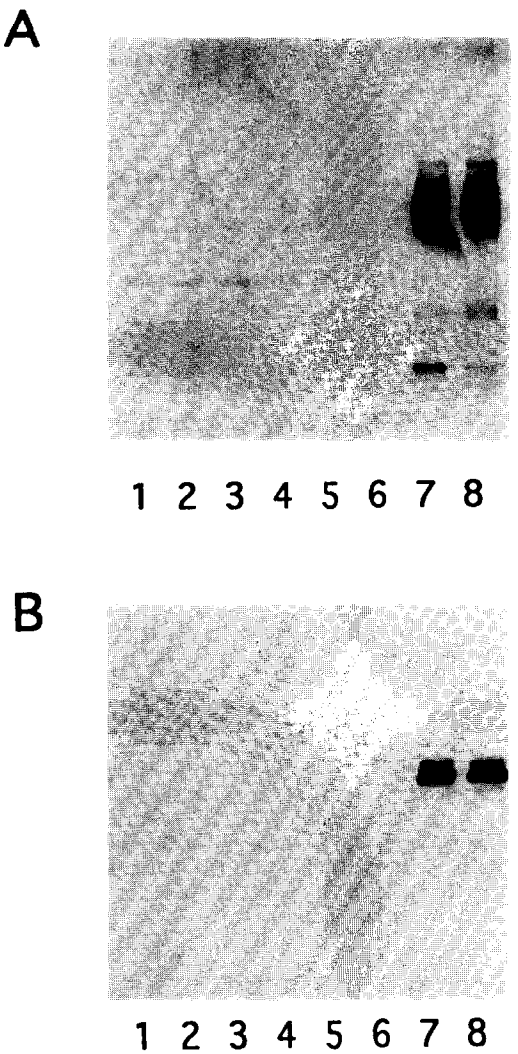

Fig. 6. Immunoreactive CYP1A1 in activated peritoneal macrophages. Macrophages were activated by treating rats on day −1 with either 5 μg poly I:C or 100 μg BCG. Animals were then gavaged with TCDD on day 0 and killed on day 4. Macrophage activation was confirmed by monitoring the reduction of 5′-nucleotidase activity. Microsomes from macrophage preparations or matching liver were prepared, electrophoresed, and blotted as described in Materials and Methods. Twenty micrograms of macrophage microsomes or 2 μg liver microsomes was applied to each lane. (A) Poly I:C. (B) BCG. Lanes 1–4 = peritoneal macrophages. Lanes 5–8 = liver. Lanes 1 and 5 = nonactivated/control. Lanes 2 and 6 = activated/control. Lanes 3 and 7 = nonactivated/TCDD-treated. Lanes 4 and 8 = activated/TCDD-treated.

Table 3. Ethoxyresorufin-*O*-deethylase activity after macrophage activation

Cell population	EROD activity* (pmol/mg/min)
Peritoneal macrophages	
Nonactivated/Control	14.8 ± 4.2†
BCG-activated/Control	12.5 ± 3.8
Nonactivated/TCDD-treated	14.5 ± 4.0
BCG-activated/TCDD-treated	12.8 ± 3.2
Whole liver	
Nonactivated/Control	212.8 ± 54.5
BCG-activated/Control	244.2 ± 55.9
Nonactivated/TCDD-treated	6518.3 ± 297.5
BCG-activated/TCDD-treated	6283.8 ± 869.6

* F344 rats were activated *in vivo* by i.v. injection of 100 μg of Bacillus of Calmette and Guerin, *Mycobacterium tuberculosis* (BCG) on day −1. Animals were then treated with corn oil or TCDD on day 0 and killed on day 4. Microsomes were isolated from macrophage or tissue preparations, and EROD activity was measured by fluorometry as described in Materials and Methods.

† Mean ± SEM of quadruplicate determinations.

stitutive and inducible enzyme. This is in contrast to several previous reports indicating that peritoneal macrophages and/or monocytes demonstrate inducible AHH activity [18, 42, 48]. Differences in procedures for isolation and purification of the cells may account for this discrepancy, as several of these investigators used longer adherence times than those used in the present studies, which would alter the activation state of the macrophages. The maturity and activation state of isolated macrophages have been shown to affect both the amount and activity of cellular enzymes including cyclooxygenases, lipoxygenases, acid hydrolases, peroxidases, and lysozyme [49, 50]. In addition, transcriptional regulation of immunomodulatory factors has been shown to vary with activation and/or differentiation in macrophages [51].

The inability of either BCG or poly I:C to induce CYP1A1 activity in peritoneal macrophages can be due to multiple factors. Various cytokines and growth factors produced by macrophages, including interleukin-1 and interleukin-6, alter cytochrome P450-dependent drug metabolism [52–55]. *In vivo* treatment with non-specific immunomodulators, such as BCG or poly I:C, induces expression and release of these cytokines. Alternatively, the differences may result from the cellular origin or the tissue milieu in which differentiation occurs. For example, it has been suggested that specific macrophage populations may arise from distinct lineages of cells from within the bone marrow or via proliferation within specific tissues [28, 56, 57].

Taken together these studies indicate that selected drug-metabolizing enzymes are present in all leukocytes, whereas others are expressed only in specific cell populations. Elucidation of the metabolic capabilities of specific populations of immune cells should assist in explaining the differential toxicity of certain xenobiotics, such as PAHs. In addition, understanding the differing capabilities of immune cells to metabolize xenobiotics to toxic intermediates will help clarify our knowledge of the mechanisms of immunotoxicity. As human peripheral blood leukocytes are readily available for testing, comparative studies with purified cell populations will allow for a better correlation between human and animal data in risk assessment.

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