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Fingerprinting of Cytochrome P450 and Microsomal Epoxide Hydrolase Gene Expression in Human Blood Cells

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To examine the character and variability of human cytochrome P450 (CYP) and microsomal epoxide hydrolase (mEH) gene expression in human blood cells, we used a highly sensitive, quantitative, competitive reverse transcriptase-coupled polymerase chain reaction (QC RT-PCR) assay to assess mRNA profiles for a battery of 8 genes, in peripheral lymphocytes isolated from 10 healthy donors. Of the genes profiled, in lymphocytes CYP2D6 was typically expressed at the highest levels (3.8×10^5 molecules/μg total RNA), with CYP2E1 and mEH also maintained at relatively high abundance (1.2×10^5 and 1.8×10^5 molecules/μg total RNA, respectively). CYP1A1 levels were approximately an order of magnitude lower (3.9×10^4 molecules/μg total RNA), followed by CYP2F1 and CYP3A levels that were near the detection limit of the assay. CYP1A2 and CYP2A6/7 mRNAs were not detected in any of the lymphocyte samples. Overall, relatively low levels of inter-individual variation (2- to 6-fold) existed among these endpoint parameters in the subjects tested. To test whether established human blood cell lines were suitable models to assess basal expression and chemical induction responsiveness of these genes, we determined that constitutive CYP and mEH mRNA profiles were essentially conserved across 4 established human blood cell lines, and highly analogous to the basal expression patterns identified in freshly isolated peripheral lymphocytes. mEH protein was detected in all of the cell lines using Western immunoblotting and chemiluminescent visualization, whereas CYP1A1, CYP2D6, CYP2E1 or CYP3A proteins were not detected in these analyses. When blood cell-derived cultures were exposed to the prototypical CYP1A and CYP3A inducers, i.e., β-naphthoflavone (β-NF), dexamethasone (DEX) or phenobarbital, generally little or no inductive response was manifested. Thus, the data obtained from this investigation indicate that, although human blood cell lines in general exhibit poor responsiveness to prototypical inducer exposures, the constitutive patterns of CYP and mEH expression in peripheral lymphocytes appear to exhibit relatively low levels of variation among individuals. In addition, these *in vivo* patterns of expression are well maintained in established cultured blood-cell lines.

Key Words: **biomarkers; blood cells; cytochrome P450 (CYP); human quantitative competitive reverse transcriptase coupled polymerase chain reaction (QC RT-PCR) assay.**

The cytochrome P450 (CYP) superfamily of genes encodes an array of monooxygenases involved in the biotransformation of a wide variety of endogenous and exogenous compounds (Gonzalez *et al.,* 1993; Nelson *et al.,* 1996; Omiecinski *et al.*, 1999). Xenobiotic modulation of CYP gene expression may substantially impact substrate metabolism and related toxicities, and therefore a "read-out" of CYP expression profiles may offer a sensitive and mechanistically based biomarker of chemical exposure (Sewall *et al.,* 1995; Walker, 1998).

While the liver is the major organ involved in biotransformation, many drug-metabolizing enzymes also are present within extrahepatic tissues, including human peripheral lymphocytes (Raucy *et al.,* 1999). Since blood is a readily accessible tissue, an appealing concept is to use peripheral blood cells as a surrogate, or sentinel model for CYP activities that manifest in internal organs. In fact, several previous studies have attempted to study the suitability of CYP expression and induction in human lymphocytes for use as a biomarker (Carcillo *et al.,* 1996; Cosma *et al.,*1992; Jacquet *et al.,*1997; Raucy *et al.,*1997; Rojas *et al.,*1992; Rumsby *et al.,*1996; Spencer *et al.,*1999; Van den Heuvel *et al.,*1993).

A number of investigators have examined the correlation between CYP1A1 enzyme activity and induction (assessed as aryl hydrocarbon hydroxylase [AHH] or ethoxyresorufin-*O*-deethylase [EROD] activity) in mitogen-stimulated human lymphocytes and lung tissue, especially with respect to the potential relationship between CYP1A1 induction by exposure to polycyclic aromatic hydrocarbons (PAHs) in cigarette smoke and the susceptibility to lung cancer (Jacquet *et al.,* 1997; Karki *et al.,* 1987; Kiyohara *et al.,*1998; McLemore *et al.,* 1978; Paigen *et al.,* 1977; Ward *et al.,* 1978). The results from these studies have been rather inconsistent. For example, Rojas and coworkers (1992) assessed CYP-dependent formation of benzo[*a*]pyrene-tetrols in lung microsomes and cultured lymphocytes and found no correlation between the two tissues in subjects with different smoking habits, whereas some modest effects of PAH exposure on CYP1A1 mRNA expression in fresh human lymphocytes have been reported (Cosma *et al.,*1992; Rumsby *et al.,*1996; Van den Heuvel *et al.,*1993). Results of other recent investigations suggested that the mRNA concentrations of two CYP enzymes, CYP2D6 and CYP2E1, in human blood lymphocytes reflect the *in vivo* activity of their

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corresponding liver counterparts (Carcillo *et al.,* 1996; Raucy *et al.,* 1997). A study examining the expression of another biotransformation enzyme, microsomal epoxide hydrolase (mEH), indicated that basal activity levels in fresh lymphocytes were correlated with levels in liver and in lung (Omiecinski *et al.,* 1993).

In order for lymphocytes or other sentinel-cell types to serve as a general surrogate for CYP gene expression in the liver or other target organs, it is necessary that the levels and patterns of expression correlate between the respective cell or tissue sets. Historically, it has been difficult to accurately monitor biotransformation systems in extrahepatic cell types, since the typically low levels of their associated expression require highly sensitive and specific assays to enable their detection. In this study, we used an extremely sensitive quantitative-competitive (QC) RT-PCR assay that allowed measure of a battery of CYP and mEH gene expression levels in peripheral blood cells, established cell lines, and their direct comparison to absolute levels obtained previously from human liver (Andersen *et al.,* 1998). We report that constitutive CYP and mEH expression profiles in blood lymphocytes are quite distinct from patterns characteristic of the liver.

MATERIALS AND METHODS

Cell Lines and Antibodies. The following human cell lines were obtained from the American Tissue Culture Collection: HEL 97.1.7 (erythroid), IM9 (B lymphoblastoid), HL60 (promyloid), THP1 (monocytoid), and HepG2 (hepatoma). Monoclonal antibodies for human CYP2D6 and polyclonal antisera for human CYP2E1 and rat NADPH P450 reductase were purchased from Gentest (Woburn, MA). Preparation of CYP1A1 and mEH-specific antisera was reported previously (Farin and Omiecinski, 1993). CYP3A specific antiserum was a generous gift from Dr. Paul Thomas, Rutgers University. Secondary antibodies (horseradish peroxidase conjugated IgGs) were purchased from Sigma-Aldrich (Saint Louis, MO). Phycoerythrin (PE)- and fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies specific for human CD3, CD19, and CD14 were purchased from Pharmingen (San Diego, CA).

Isolation of Peripheral Lymphocytes from Donors. Peripheral blood (40ml) was obtained by venapuncture from 10 normal healthy volunteers, 5 female and 5 male Caucasians between the ages of 22 and 56. Mononuclear cells were isolated using Ficoll density gradient centrifugation (Pharmacia, Inc., Piscataway, NJ) according to manufacturer's instructions. Isolated cells were resuspended in RPMI 1640 medium (Life Technologies Inc., Grand Island, NY) supplemented with 100 U/ml penicillin and 100 ng/ml streptomycin (Sigma-Aldrich Inc., Saint Louis, MO), placed into tissue culture flasks, and incubated at 37°C for thirty min, allowing monocytes to adhere. Following incubation, non-adherent lymphocytes were recovered from the medium. Purity of these cell populations was determined by fluorescent activated cell sorting (FACS) using a Coulter Epics Elite ESP (Coulter Corp., Miami, FL) analysis with human CD3-, CD19-, and CD14-specific antibodies to label T lymphocytes, B lymphocytes, and monocytes, respectively. The cell isolates typically contained less than 4% monocytes, about 6% B lymphocytes, and about 70% T lymphocytes.

Cell Culture and Treatments. Human blood cell lines were cultured in suspension in RPMI 1640 medium (Life Technologies) supplemented with 10% Nu-serum (Becton Dickinson Inc., Franklin Lakes, NJ), 100 U/ml penicillin, and 100 ng/ml streptomycin (Sigma-Aldrich) at 37°C and 5% CO_2. HepG2 cells were cultured in DMEM/F12 medium (Life Technologies), supplemented with 10% Nu-serum, 100 U/ml penicillin, and 100 ng/ml streptomycin at 37°C and 5% CO_2, except for one control induction experiment where they were grown in the RPMI 1640 medium. For chemical treatments, logarithmically growing cells were pre-cultured in medium supplemented with 2% Nu-serum and antibiotics for 24 h. These culture conditions were derived empirically as optimal for the cell lines in use. Cells were incubated with 22 μM β-naphthoflavone (βNF), 22 μM βNF plus 25 nM dexamethasone (βNF + DEX), 10 μg/ml Arochlor 1254 (ARO), 10 μM dexamethasone (DEX) or 1 mM phenobarbital (PB) in medium containing 2% Nu-serum. The concentrations of inducers used were taken from general literature values. Control cultures were incubated with DMSO (typically $<$0.05%). Cell viability after treatment was assessed by Trypan blue exclusion and was typically $>$85%.

RNA Isolation and Quantitative-Competitive (QC) RT-PCR Assay. Total cellular RNA was isolated from cell preparations using the Trizol Reagent (Life Technologies) following the manufacturer's instructions. The concentrations of RNA were determined spectrophotometrically by monitoring UV absorbance at 260 nm. Between 0.2 and 5 μg of total RNA were used for quantification of mRNA levels for CYP 1A1, 1A2, 2A6/7, 2D6, 2E1, 2F1, 3A, and microsomal EH, using a QC RT-PCR assay described previously (Andersen *et al*., 1998).

DNA Sequencing. Sequencing of PCR products was performed using the ABI PRISM BigDye™ DNA sequencing kit from PE Applied Biosystems (Foster City, CA). Samples were analyzed on an ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems).

Fluorescent Activity Assay. Ethoxyresorufin-O-deethylase (EROD) activity was measured in whole cell suspensions. Briefly, cells were washed and resuspended at a concentration of 10^6 cells/ml in Earle's balanced salt solution (Life Technologies) supplemented with 15 mM HEPES, 5 mM $MgCl_2$, 2.5 mM $CaCl_2$, pH 7.5, gassed with 95% O_2/5% CO_2 and supplemented with 25 mM dicumarol. Cells were kept at 37°C. The reaction was initiated by adding 1 μl of 5 mM 7-ethoxyresorufin in DMSO to a 1-ml cell suspension. Fluorescence intensity was measured over the period of 5 min and at 10-, 15-, 20-, and 30-min time points, using a wavelength set of 547 nm (excitation) and 584 nm (emission) with a Perkin Elmer LS50 fluorescent detector. Rates were calculated from the slopes in the linear range of increase in fluorescence intensity. The results were quantified using resorufin as a standard (Sidhu *et al*, 1993).

Preparation of Microsomal Proteins and Western Blot Analysis. Microsomes from cell lines were prepared by differential centrifugation. Briefly, cells were washed, resuspended, and sonicated in ice-cold homogenization buffer (10mM KH_2PO_4, 1.15% KCL). Cell homogenates were centrifuged at 9000 $\times$ *g* for 20 min. Supernatants were collected and centrifuged for 1 h at 100,000 $\times$ *g* at 4°C. The resulting pellets were resuspended in storage buffer (10 mM KH_2PO_4, 1mM EDTA, 20% glycerol). Protein contents were assessed by the BCA reagent assay (Pierce Inc., Rockford, IL).

Twenty μg of microsomal protein from each cell line and 10 μg of human liver microsomes were electrophoretically separated on 10% sodium dodecyl sulfate-polyacrylamide gels and electroblotted to Sequi-Blot PVDF membranes (Bio-Rad Inc., Hercules, CA) according to standard methods. The membranes were subsequently blocked in 1 $\times$ TTBS (10 mM Tris base, 0.9% NaCl, pH 7.4, 0.1% Tween 20) plus 1% bovine serum albumin (Boehringer Mannheim, Inc., Germany), and 1% dry milk (Bio-Rad Inc., Hercules, CA). Primary antibodies, diluted from 1:2000 to 1:10,000 were applied to the membranes for 2 h at room temperature. After 3 15-min washes with 1$\times$ TTBS, the membranes were exposed to secondary antibodies for 1 h (horseradish peroxidase-conjugated IgGs at a 1:4000 dilution) and washed as described. Chemiluminescent visualization of the resulting immunoblots was achieved with ECL system (Amersham, Inc., Arlington Heights, IL).

RESULTS

Profiles of CYP and mEH Gene Expression in Fresh Lymphocytes

To begin this investigation, we determined basal expression profiles of several biotransformation enzyme genes of human peripheral lymphocytes. Total RNA from lymphocytes, isolated from whole blood of 10 healthy volunteer donors, 5 male

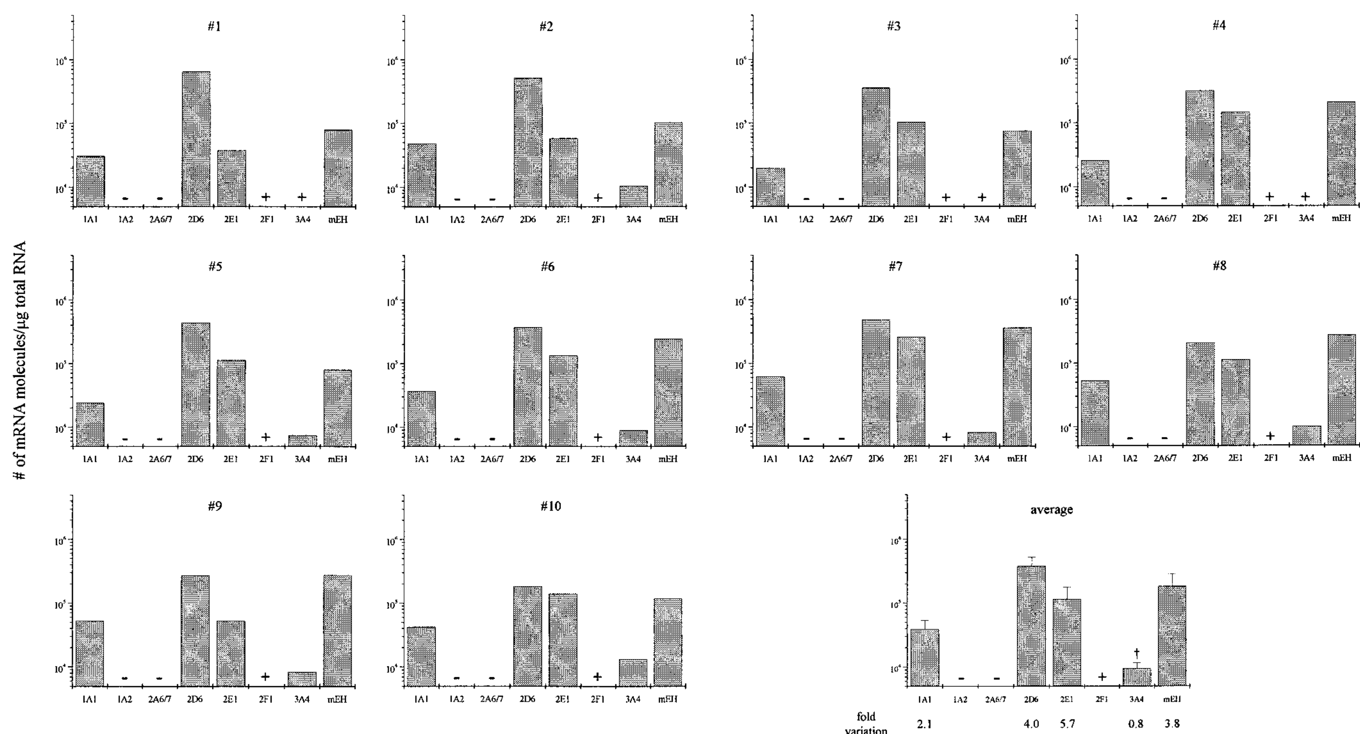

FIG. 1. Profiles of constitutive CYP and mEH mRNA levels in freshly isolated lymphocytes from 10 healthy donors. Values are means of 2 independent determinations and lymphocyte preparations obtained from the same individual at least 2 weeks apart. Typically, there was <15% intra-individual variation observed between the repeated measures. Samples #1, #4, and #6 were obtained from female subjects; samples #5 and #7–10 were from males. "+": Denotes $<5.0 \times 10^3$ mRNA molecules/μg total RNA; "–": indicates not detectable. Error bars on average values indicate standard errors of the mean. "†": Denotes average and fold variation calculated only from values greater than 5.0×10^3 molecules/μg total RNA ($n = 7$).

and 5 female Caucasians between the ages of 22 and 56, was analyzed for gene expression of 7 key cytochrome P450 enzymes and mEH, using a QC RT-PCR assay described previously (Andersen et al., 1998). These analyses were repeated from blood samples obtained from the same individuals approximately 1 month subsequent to the initial sampling. Gene expression profiles for each donor and averaged values are presented in Figure 1.

Low levels of CYP1A1 mRNA ($2.0–6.2 \times 10^4$ molecules/μg total RNA) were detected in the lymphocytes from each donor. CYP2D6 mRNA was quantified at levels ranging between 1.8×10^5 and 9.1×10^5 molecules/μg total RNA in each individual. CYP2E1 and mEH RNA expression levels varied from 3.8×10^4 to 2.6×10^5 molecules/μg total RNA and from 7.6×10^4 to 3.6×10^5 molecules/μg total RNA, respectively. CYP3A, identified specifically as CYP3A4 by means of DNA sequence analysis of the amplified products, was detectable but expressed at very low levels (approximately $0.8–1.3 \times 10^4$ molecules/μg total RNA) and could not be quantified in all samples. CYP2F1 expression was detectable in each individual but the amounts were not sufficient for quantification ($<5.0 \times 10^3$ molecules/μg total RNA). CYP1A2 and CYP2A6/7 mRNA could not be detected in any of the lymphocyte RNA samples. All values reported represent averages of measurements from 2 independent experiments, with variation typically <15% between measures.

Overall, the profiles appeared to be substantially conserved among individuals. The degree of variation among individuals ranged from 2.1-fold (CYP1A1) to 5.7-fold (CYP2E1). CYP2D6 and mEH exhibited 4.0- and 3.8-fold variations among individuals, respectively. No gender related differences in expression profiles could be ascertained. Together, these results portray a comprehensive picture of basal CYP and mEH gene-expression patterns in human peripheral lymphocytes and demonstrate an essentially low level of interindividual variation within these cell types.

Profiles of CYP and mEH Gene Expression in Human Cell Lines

To compare CYP and mEH gene expression in established human cell lines with those found in freshly isolated human lymphocytes, total RNA from the following human cell lines was subjected to analysis with QC RT-PCR: the erythroblastic leukemia cell line, HEL 92.1.7; the B lymphoblastic leukemia cell line, IM9; the promyelocytic leukemia cell line, HL60; the monocytic leukemia cell line, THP-1; and the hepatoma cell line, HepG2. Constitutive expression profiles of logarithmically growing cells are shown in Figure 2.

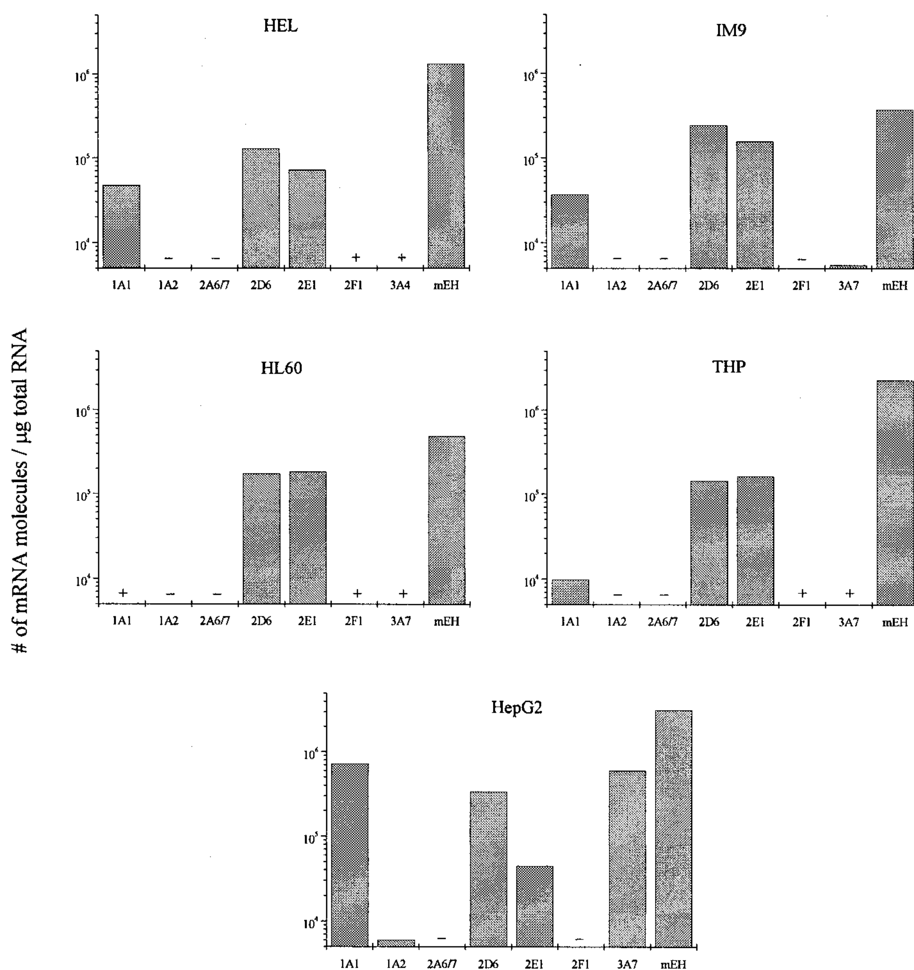

FIG. 2. Profiles of constitutive CYP and mEH mRNA levels in HEL, IM9, HL60, THP, and HepG2 cells. Values represent means of two measurements. "+": Denotes $<5.0 \times 10^3$ mRNA molecules/μg total RNA; "–": indicates not detectable.

mEH was expressed at the highest levels in all cell lines tested (3.68×10^5–3.18×10^6 molecules/μg total RNA). Among the blood cell lines, mEH also exhibited the highest degree of variation (5.7-fold). CYP2D6 and CYP2E1 gene expression levels were relatively high (7.20×10^4 – 2.35×10^5 molecules/μg total RNA) and conserved among blood-derived cells (0.8- and 1.6-fold variation, respectively). Interestingly, of the 8 gene products quantified, CYP2E1 was the only RNA expressed at higher levels in the blood cells than in hepatoma cells. The levels of CYP1A1 RNA were low in all hematopoietic cells. In the blastoid cells, HEL and IM9, 4.75×10^4 and 3.67×10^4 molecules/μg total RNA were quantified for CYP1A1. In HL60 and THP cells, both representing later stages of cell differentiation, CYP1A1 mRNA was marginally detectable (1.0×10^4 and 5.0×10^3 or less molecules/μg total RNA, respectively). CYP3A was highly expressed in HepG2 hepatoma cells but was barely detected in any of the blood cell lines. By means of DNA sequencing we determined that HEL cells specifically express CYP3A4, whereas all other cell lines express CYP3A7 (data not shown). Traces of CYP2F1 message were detected only in HEL, IM9 and THP cells, whereas CYP1A2 was detected only in HepG2 cells. CYP2A6/7 was not detected in any of the cell lines. All values presented are average of measurements from at least 2 independent experiments, with <15% variation between repeated analyses.

Overall, profiles of CYP and mEH basal gene expression were largely conserved among cells from different lineages and the differentiation stage of human hematopoiesis. Moreover, both the levels and the profiles of gene expression, measured in all the blood cell lines, resembled those exhibited in freshly isolated lymphocytes. The latter observation indicates that these established blood cell lines possess a set of basic features resembling that of freshly isolated lymphocytes and therefore may serve as suitable *in vitro* models for xenobiotic biotransformation in human blood.

Inducibility of CYP1A and CYP3A Gene Expression in Human Cell Lines

To test whether human blood cell lines are capable of induction responses subsequent to xenobiotic challenge, we cultured the various lines in the presence of several prototypical inducers and examined the resulting mRNA expression levels.

For evaluation of CYP1A gene responsiveness, logarithmically growing cells were treated with DMSO (vehicle control), 22 μM βNF, 22 μM βNF + 25nM DEX, or 10 μg/ml ARO,

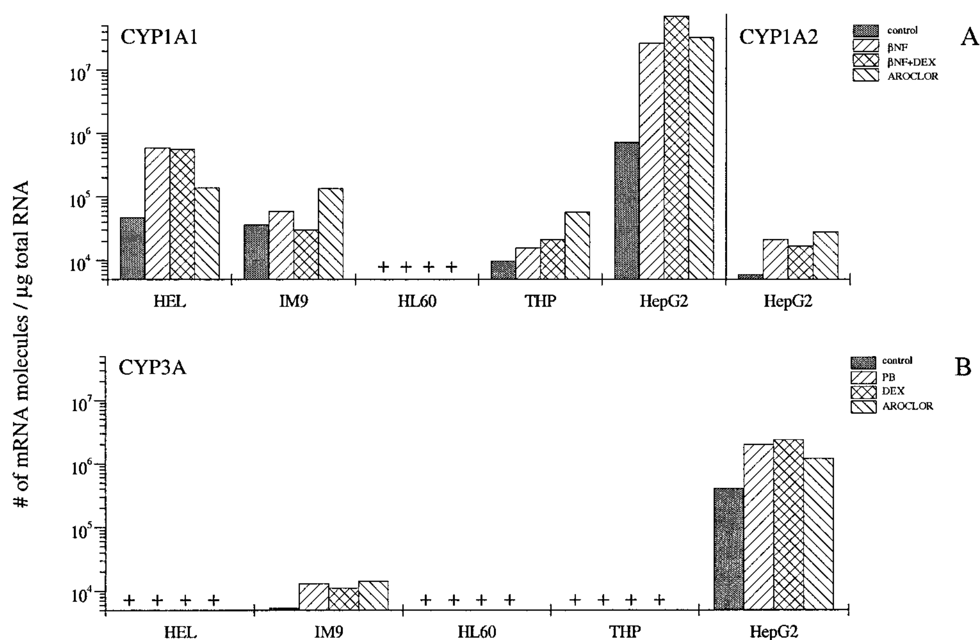

FIG. 3. Inducibility of CYP1A and CYP3A mRNA in HEL, IM9, HL60, THP, and HepG2 cells. (A) RNA isolated from cells treated for 24 h with DMSO (vehicle control), 22 M βNF, 22 μM βNF + 25nM DEX, or 10 μg/ml ARO, respectively, was analyzed for CYP1A1 and CYP1A2 gene expression using QC RT-PCR. CYP1A2 was not detected in HEL, IM9, HL60, or THP cells after any treatment (data not shown); levels detected in HepG2 cells are indicated on the inset graph. "+": Indicates $<5.0 \times 10^3$ mRNA molecules/μg total RNA. (B) RNA isolated from cells treated for 24 h with DMSO (vehicle control), 1.5 mM PB, 10 μM DEX, or 10 μg/ml ARO, respectively, was analyzed for CYP3A gene expression. "+": Indicates $<5.0 \times 10^3$ mRNA molecules/μg total RNA.

respectively, for 24 h. Total RNA was isolated from these cells and analyzed for CYP1A1 and CYP1A2 gene expression using QC RT-PCR. The results are summarized in Figure 3A.

Among all the blood cell lines used in this study, HEL was the only line exhibiting substantive CYP1A1 RNA responsiveness, with 12-fold increases detected above basal expression following treatment with βNF and βNF + DEX. Chemical challenge increased CYP1A1 mRNA levels in IM9 and THP cells only slightly (approximately 2- to 3-fold). HL60 cells exhibited barely detectable levels of CYP1A1 mRNA and neither treatment increased expression levels above 5.0×10^3 molecules/μg total RNA. CYP1A2 was not detected in any of the blood cell lines after any of the inducer treatments tested (data not shown). In contrast, CYP1A1 gene expression increased approximately 45-fold in HepG2 cells after treatment with βNF and ARO, and approximately 100-fold after treatment with βNF+DEX. The effects of these chemical inducers on CYP1A2 expression in HepG2 cells were substantially weaker (3- to 5-fold induction).

To examine induction of CYP1A1 enzyme activity, EROD assays were performed on DMSO and βNF + DEX (48 h) treated cells. However, blood cell line, control or induced, exhibited EROD activity higher than the background of the assay (data not shown). In contrast, induced HepG2 cells exhibited relatively large increases in EROD levels, 10-fold higher than controls (data not shown), reflective of the large increases of CYP1A1 mRNA measured independently.

For evaluation of CYP3A gene responsiveness, logarithmically growing cells were treated with DMSO (vehicle control), 1.0 mM PB, 10 μM DEX or 10 μg/ml ARO, respectively, for 24 h. Total RNA was isolated from the cells and analyzed for CYP3A gene expression using QC RT-PCR. The results are summarized in Figure 3B.

Constitutive CYP3A gene expression in HEL, HL60, and THP cells was very low (less then 5.0×10^3 molecules/μg total RNA) and this level was not increased subsequent to treatment with the 3 prototypic inducers assayed. A slight increase in CYP3A, reflecting approximately 2.5-fold induction, was detected in IM9 cells after chemical challenge. The constitutive CYP3A7 mRNA level in HepG2 cells was relatively high and increased moderately (approximately 3- to 5-fold) after treatments. All values presented were an average of measurements obtained from at least 2 independent experiments, with <15% variation between replicate analyses. Induction experiments performed using serum-free culture conditions or extending treatments for 48 h did not result in altered cellular responsiveness (data not shown).

Overall, the blood cell lines tested in this study exhibited only weak responsiveness to prototypic inducers of CYP1A and CYP3A. Therefore, their use as models to assess the response of chemical exposure in humans or to study mechanistic aspects of xenobiotic CYP gene induction occurring in the liver appears limited.

Protein Expression in Human Cell Lines

To determine functional levels of CYP and mEH protein, microsomes were isolated from all the cell lines described above and were subjected to analysis by Western immunoblotting. Antibodies specific for CYP1A1, CYP2D6, CYP3A, CYP2E1, mEH, and P450 reductase were assessed in these studies. The results of these experiments are presented in Figure 4.

Of all the proteins examined, only mEH and P450 reductase were detectable in the cell lines. HepG2 cells appeared to possess much higher levels of these proteins as compared to the

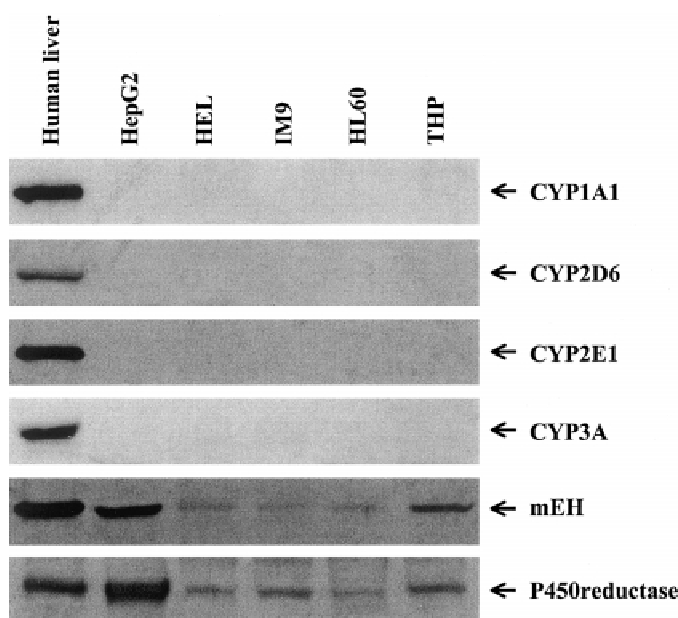

FIG. 4. Western blot analysis of HepG2, HEL, IM9, HL60, and THP cells. Twenty μg of microsomal protein isolated from the respective cell lines and 10 μg of protein from a human liver microsomal preparation were loaded in each sample well. The blots were probed with specific antibodies against CYP1A1, CYP2D6, CYP3A, CYP2E1, mEH, and P450 reductase, as described in Materials and Methods.

blood-derived cells. Human liver microsomes were used as a positive control for these studies and demonstrated strong signals for all the proteins undergoing analysis. The liver samples used to prepare these microsomes were previously shown to express high levels of the specific CYPs and mEH mRNAs (unpublished data). A P450 reductase-specific antibody was used to ascertain the integrity of the microsomal protein fractions. It is noteworthy that all of the blood-derived cells possessed lower levels of P450 reductase than did hepatoma or human liver cells.

These results demonstrate the existence of mEH protein in human blood cell lines but suggest that the level of CYP biotransformation enzymes is, at best, extremely low. Correlating well with the positive results of the Western blot signals, mEH mRNA levels were similarly of highest abundance in these sample preparations.

DISCUSSION

The potential utility of human blood cells as biomarkers of target-organ susceptibility, or as markers of chemical effects subsequent to exposure scenarios, remains a desirable goal for researchers in toxicology. For example, monitoring of blood lymphocyte activity and/or genetic status has been shown to be of value in assessing thiopurine *S*-methyltransferase activity status and individualizing pharmacotherapy with thiopurine compounds (Krynetski and Evans, 1998; Otterness *et al.*, 1998). We conducted the present investigation to evaluate the suitability of human blood cells as biomarkers of oxidative biotransformation. In this regard, we believe this study represents the first comprehensive analysis of gene expression profiles for a battery of targeted biotransformation enzymes in human peripheral lymphocytes and in established human blood cell lines. To facilitate these investigations, we adopted a QC RT-PCR assay (Andersen *et al.*, 1998), used previously to assess comparative CYP and mEH expression profiles across a spectrum of human liver samples. The results obtained from these studies reveal striking differences between inherent liver and lymphocyte CYP expression profiles.

Initial studies were conducted using fresh lymphocyte preparations from 10 individual donors. The studies were repeated with separate blood samples obtained approximately 1 month later from the same individuals. Overall, we observed a remarkably low inter-individual variation of CYP and mEH gene expression in lymphocytes, ranging from 2- to 6-fold in our sample of 10 individuals. To date, most studies have indicated less than 10-fold inter-individual variation of basal expression of CYP genes in freshly isolated lymphocytes (Dassi *et al.,* 1998; Carcillo *et al.,* 1996; Van den Heuvel *et al.,* 1993). In contrast, in our previous analysis of 8 human livers, the variation in RNA expression ranged from 13-fold (CYP2E1) to 220-fold (CYP2D6), likely explained by a combined set of factors including genetic polymorphism and possible influences of nutritional deprivation, medication, and ischemic conditions during organ harvest (Andersen *et al.,* 1998).

More specifically, CYP1A1 mRNA was detectable in the lymphocyte preparations from all 10 of the individual donors, albeit at low levels (Fig. 1). This CYP was undetected in 5 of 8 human liver samples using the same highly sensitive assay procedure (Andersen *et al*., 1998). The presence of CYP1A1 mRNA in fresh lymphocytes also has been reported in other studies (Van den Heuvel *et al.,* 1993; Lang *et al.,* 1998; Omiecinski *et al.,* 1990; Rumsby *et al.,* 1996 and Wei *et al.,* 1998, 1999). In contrast, CYP1A2, like CYP2A6/7, was highly abundant in liver (Andersen *et al.,* 1998) but was not detectable in fresh lymphocytes (Fig. 1). This latter finding appears to be in agreement with previous reports (Hukkanen *et al*. (1997), Koskela *et al*. (1999) and Raunio *et al*. (1998)).

It is noteworthy that we observed only very low abundance of CYP3A mRNA in fresh lymphocytes (Fig. 1), near the detection limits of our assay. However, through DNA sequence analysis, we clearly identified CYP3A4 as the specifically expressed CYP3A form in the lymphocyte-derived PCR products. It was reported recently that CYP3A is selectively expressed only in B lymphocytes (Sempoux *et al.,* 1999). This information may explain the generally low levels of CYP3A detected in our cell preparations since they contained only about 6% of this cell type as assessed by FACS analysis (see Materials and Methods). In contrast, Janardan and coworkers (1996) were unable to detect CYP3A4 mRNA in mononuclear blood cell preparations, although they did report detection of CYP3A5-specific mRNA in one out of 6 individuals. It should be emphasized that the CYP3A-selective forward and reverse

primers used in this investigation, and Andersen's study (1998), exhibit perfect homology with endogenous CYP3A4 and CYP3A7 sequences but mismatch slightly (2/20 and 2/22 bases, respectively) with the corresponding CYP3A5 sequence and thus may have affected our ability to robustly amplify CYP3A5 sequences. In comparison, it appears that all human adult livers express CYP3A4, whereas CYP3A5 and CYP3A7 are present in only about 25% of all postnatal livers (Aoyama *et al.,*1989; Schuetz *et al.,*1994; Wrighton *et al.,*1989, 1990).

CYP2F1 is considered a lung specific protein (Nhamburo *et al.,* 1989), expressed in bronchial epithelial cells and in alveolar macrophages (Hukkanen *et al.,* 1997; Willey *et al.,* 1996). Therefore, the weak CYP2F1 signals in our samples may be ascribed to the monocytic population (approximately 2.4%) in our cell preparation. Although CYP2D6 and mEH mRNAs are expressed at relatively high levels in lymphocytes, they are still approximately one-tenth as abundant as that in the human liver (Andersen *et al.,* 1998). The difference for CYP2E1 is even more dramatic, with lymphocytes expressing approximately 1/580 the levels of liver (Andersen *et al.,* 1998).

Taken together, we conclude that with respect to expression of phase I biotransformation enzymes, human liver and peripheral lymphocytes are rather distinctly equipped, both with respect to overall levels as well as inherent profiles of gene expression. This result suggests that the applicability of blood cells as surrogates for liver biotransformation capacities should be evaluated cautiously.

Additional objectives of this investigation were to test whether established human blood cell lines could appropriately model basal expression occurring in primary blood lymphocytes, and to assess whether xenobiotic induction responses of blood cells may serve as a biomarker of induction responses occurring in the liver. Human leukemia cell lines express major blood cell-specific markers and are widely used models for the study of hematopoesis, cell differentiation, and CYP expression/regulation (Auwerx, 1991; Collins 1987; Jakob *et al*., 1995; James *et al*., 1999; Masten *et al*., 1996). Our data demonstrate that, with respect to constitutive CYP and mEH RNA levels, the established cell lines we studied exhibited expression character that resembled freshly isolated lymphocytes remarkably well (Fig. 2). This result is somewhat surprising considering the significant differences in profiles between human liver (Andersen *et al.,* 1998) and the hepatoma HepG2 cells (Fig. 2). Interestingly, no substantive differences in CYP and mEH profiles were noted among the representatives of the different lineages of human hematopoesis, i.e., erythrocytic, lymphocytic, mylocytic, or monocytic cell line derivatives (Fig.2).

With respect to induction, of the 4 blood-cell lines analyzed, only HEL cells exhibited considerable CYP1A1 gene responsiveness to the prototypical inducers βNF and ARO (Fig. 3A). Although we attempted EROD activity measurements on all the cell lines, it was curious that none of the lines, including HEL cells, exhibited EROD levels that were distinguishable from background controls (data not shown). Perhaps this result attests to the sensitivity of the QC RT-PCR assay used here. In comparison, using HepG2 cells, βNF treatment resulted in 100-fold induction in CYP1A1 mRNA levels that paralleled a 10-fold increase in induced EROD activity (data not shown). The presence of the aryl hydrocarbon receptor (AhR) and the aryl hydrocarbon receptor nuclear translocator protein (ARNT), which are prerequisites for CYP1A1 induction, have been demonstrated in HEL, IM9, HL60, and THP cells (Hayashi *et al.,* 1995; Masten *et al.,* 1995). However, Hayashi *et al*. (1995) also demonstrated a positive correlation between the stage of cell differentiation, expression level of AhR and induction of CYP1A1 gene expression for several human leukemia cell lines. Thus, although HL60 cells expressed high levels of AhR, they were functionally responsive to xenobiotics only after treatment with stimulators of monocytic differentiation (Hayashi *et al.,* 1995). Similarly, blood lymphocytes exhibit CYP1A1 gene induction only subsequent to stimulation with mitogens (Kouri *et al.,* 1974). In mitogen-stimulated human blood lymphocytes, CYP1A1 mRNA expression is induced 10- to 20-fold by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a response that is also correlated with induction of EROD activity (Van den Heuvel *et al.,* 1993; Lang *et al.,* 1998).

In addition to the low CYP1A1 responsiveness, the lack of CYP3A inducibility (Fig. 3B) is a further indication of the limited suitability of these cell lines to function as appropriate models to study hepatic responses or mechanistic aspects of xenobiotic CYP gene induction, or to assess the risk of chemical exposure in humans. Interestingly, our PCR product DNA sequence analysis indicated that only the HEL cells expressed CYP3A4, the adult-specific isoform of this gene subfamily. This finding, coupled with the observation that HEL cells exhibit CYP1A1 inducibility, suggest that these cells may reflect a more advanced stage of differentiation than the other cell lines. In IM9, HL60, and THP cells, using sequence analysis, we specifically identified CYP3A7, the fetal form of CYP3A that is also found in dedifferentiated HepG2 hepatoma cells (Schuetz *et al.,* 1993 and 1994).

Finally, in this study we investigated the correlation between mRNA and protein levels. Western blot analysis revealed that CYP proteins are present in very low abundance in these cell lines (Fig. 4). As a comparison, in human lymphocytes, constitutive CYP3A protein was detectable (Janardan *et al.,* 1996; Sempoux *et al.,* 1999, Starkel *et al.,* 1999), whereas CYP2E1 protein was detectable only under inducing conditions (e.g., in diabetic patients) (Song *et al.,* 1990). However, the relatively high abundance of mEH mRNA correlates with detectable mEH protein levels in all the cell lines (Fig. 4) suggesting that a mRNA level of 5×10^5 copies per μg total RNA reflects the approximate detection limit for corresponding protein levels. Again, these results likely attest to the extremely sensitive nature of detection afforded by the QC RT-PCR assay used in this investigation.

In summary, given the relatively low levels of both mRNA and protein expression in blood cells associated with a battery

of selected drug metabolizing enzymes, we conclude that the blood-cell lines characterized in this study have only limited value with respect to modeling the basal or inducible gene expression character of biotransformation in the liver, or, in this sense, as a suitable biomarker of assessing risk of chemical exposure in humans. However, the QC RT-PCR assay used in this study was demonstrated to be a powerful tool to characterize different cell types for their respective CYP gene-expression patterns and induction behavior. Our laboratory is currently extending and adapting this type of quantitative assay for the Taqman™-based real time-PCR technology to create an even more powerful tool for quantitative analysis of gene expression. Although the effectiveness of blood cells as models of liver expression is called into question by these studies, importantly, our data do suggest that established human blood cell lines reflect the constitutive CYP and mEH expression status of primary blood lymphocytes, and therefore, are appropriate models for studying blood cell-related xenobiotic metabolism. Since the biotransformation properties of lymphocytes, as well as many other nonhepatic cells, importantly contribute to cell-specific and tissue-specific toxic events, it is desirable to more precisely assess these contributions, using sensitive means of detection such at those used in the current investigation.

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