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Cytochrome P450 1B1: A Major P450 Isoenzyme in Human Blood Monocytes and Macrophage Subsets

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ABSTRACT. In this study, cytochrome P450 (*CYP*; EC 1.14.14.1)-dependent activities and P450 isoenzyme patterns were determined in human monocytes and macrophages, which play a major role in antigen processing including small molecular weight compounds which cause contact dermatitis or drug-allergic reactions. Using reverse transcriptase-polymerase chain reaction (RT-PCR) we determined the mRNA expression of eight *CYP*s (1A1, 1A2, 1B1, 2B6/7, 2E1, 3A3/4, 3A7 and 4B1) in human blood monocytes and macrophage subsets 27E10 and RM3/1. To study the influence of known P450 inducers, monocytes were incubated in vitro with ethanol, dexamethasone, cyclosporin A (CSA), benzanthracene (BA), phenobarbital (PB), lipopolysaccharide (LPS) and 12-O-tetradecanoyl-phorbol-13-acetat (TPA) for 24 hr. Percoll density gradient isolated monocytes as well as the pro-inflammatory macrophage subtype 27E10 expressed 1B1, 2E1 and 2B6/7. On the other hand, in the anti-inflammatory macrophage subtype RM3/1, predominantly 1B1 and to some extent 2B6/7 were found. Treatment with cyclosporin A, phenobarbital, benzanthracene or ethanol resulted in induction of the expression of 3A3/4. *CYP*1B1 was the predominant isoenzyme in all monocytes and macrophages. In monocytes purified by adherence or induced by benzanthracene, lipopolysaccharide or 12-O-tetradecanoyl-phorbol-13-acetat, 1A1 was also expressed. Northern blot analysis confirmed the presence of *CYP*1B1 in monocytes and macrophages, a presence which was also demonstrated on the protein level by immunoblot and by immunohistochemical staining of the cells. The expression of several *CYP*s in monocytes/macrophages suggests that these cells may be important in the metabolism of small molecular weight compounds, which play a role in allergic contact dermatitis and drug reactions. Of particular interest is the remarkably strong expression of the recently identified dioxin inducible *CYP*1B1, known to be present in a wide range of malignant tumors. BIOCHEM PHARMACOL **56**;9:1105–1110, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. monocytes; macrophages; cytochrome P450; *CYP*1B1

*CYP*s[‖] (EC 1.14.14.1) are a multigene family of constitutive and inducible enzymes that play a central role in the metabolic activation and detoxification of various xenobiotics, including small molecular weight compounds that cause allergic reactions. Many allergic reactions which are elicited by such agents, including drug allergy or allergic contact dermatitis, are induced by small molecular weight compounds, which require covalent binding to high molecular compounds such as proteins in order to become antigenic according to the generally accepted hypothesis of Landsteiner [1]. Studies from our laboratory showed that the addition of murine liver microsomes with P450-dependent catalytic activity to several drugs, prior to their addition to lymphocytes from patients suffering from allergic reactions to these drugs, are able to increase the stimulatory effect of these cells in vitro [2]. Recently, it has been observed that only mice with inducible *CYP*1A1 were able to develop an allergic response to eugenol [3]. A major role in antigen processing is played by the antigen-presenting cells such as monocytes or macrophages; however, the knowledge concerning the xenobiotic-metabolizing capacity of these cells is rather limited [4, 5]. In the present study, we assessed *CYP* isoenzyme expression at the mRNA and protein levels in peripheral blood monocytes and macrophages. Furthermore, we were interested to define whether functional different macrophage subsets vary in their P450 isoenzyme pattern.

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‖ *Abbreviations*: BA, benzanthracene; BP, benzopyrene; CSA, cyclosporin A; CYP, cytochrome P450; LPS, lipopolysaccharide; PB, phenobarbital; RT-PCR, reverse transcriptase-polymerase chain reaction; SSC, sodium sodium chloride citrate; and TPA, 12-O-tetradecanoyl-phorbol-13-acetat.

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MATERIALS AND METHODS

Peripheral Blood Monocytes

Human peripheral blood monocytes were isolated from buffy coats derived from healthy donors (Institute for Transfusionmedicine) with a Ficoll–Paque gradient fol-

TABLE 1. Primers used for PCR analysis [13]

CYP	Sense primer location	Antisense primer location	PCR product
1A1	TCACAGACAGCCTGATTGAGA 928–947	GATGGGTTGACCCATAGCTT 1341–1360	433
1A2	TGGCTTCTACATCCCCAAGAAAT 1199–1221	TTCATGGTCAGCCCCGTAGAT 1488–1507	309
1B1	GTATATTGTTGAAGAGACAG 2423–2442	AAAGAGGTACAACATCACCT 2719–2738	316
2B6/7	CCATACACAGAGGCAGTCAT 1045–1064	GGTGTCAGATCGATGTCTTC 1402–1421	377
2E1	AGCACAACTCTGAGATATGG 925–944	ATAGTCACTGTACTTGAACT 1271–1290	366
3A3/4	CCAAGCTATGCTCTTCACCG 1279–1298	TCAGGCTCCACTTACGGTGC 1583–1602	324
3A7	CTATGATACTGTGCTACAGT 1041–1060	TCAGGCTCCACTTACGGTCT 1496–1515	475
4B1	TGACCATGTGCATCAAAGGAG 1109–1128	AAAGCCATTCTTGGAGCGCA 1487–1506	398
β-Actin	ACCCACACTGTGCCCATCTA 488–507	CGGAACCGCTCATTGCC 761–777	290
GAPDH	CCACCCATGGCAAATTCCATGGCA 212–235	TCTAGACGGCAGGTCAGGTCCACC 786–809	598

lowed by hypotonic density centrifugation in Percoll [6, 7] or by plastic adherence [8], resulting in a purity greater than 80% as judged from cytospin stained by nonspecific esterase. The cells were cultured in hydrophobic Teflon bags in RPMI 1640 with 10% human serum as described [7]. The macrophage subsets 27E10 [9] and RM3/1 [10] were isolated from 2-day cultures using an indirect immunomagnetic procedure employing the MACS microbeads and leading to a purity of >95% of either subset [11]. Expression of P450 isoenzymes was investigated in freshly isolated monocytes or monocytes cultured in the presence of various known P450 inducers in vitro for 24 hr and in monocytes cultured for 1 to 7 days. The effect of the following compounds was determined: ethanol (0.1 M), dexamethasone (10^{-6} M), CSA (10^{-6} M), BA (10^{-6} M), PB (0.4 M), LPS (1 μg/mL) and TPA (10^{-8} M). For Northern blot analysis and immunoblot, only freshly isolated monocytes were used.

RNA Isolation

mRNA was extracted from 2×10^6 cells with the Oligotex Direct mRNA-purification kit (Qiagen) using the mRNA-enrichment protocol [12]. Total RNA from monocytes and macrophages was isolated using the RNeasy Total RNA Kit (Qiagen) as per manufacturer's protocol.

RT-PCR

Reverse transcription and PCR was performed with the GeneAmp RNA PCR kit (Perkin Elmer) according to the manufacturers instructions. Detection of specific mRNAs for P450 isoforms was achieved by using primers designed to amplify at least one intron in the gene to exclude contamination of cDNA with genomic DNA [13]; GAPDH was used as an internal standard (Table 1). Amplification was carried out with 35 cycles of 1-min denaturation at 93°, 1-min annealing at 52° and 1-min extension at 72°. Amplification was terminated with an extension step of 5-min duration after the last cycle. PCR products were separated on 1.0% agarose gels (1× TBE) and stained with ethidium bromide.

Northern Blot Analysis

Ten micrograms of total RNA or 2 μg of mRNA were separated on a 1% denaturing agarose gel and transferred to Nitro Plus membranes (MSI). The filters were prehybridized at 42° for more than 6 hr in 100 mM of $NaPO_4$ -buffer pH 6.5, 10× SSC, 0.04 Denhardts and 2% glycine. The hybridisation was performed in 40 mM of $NaPO_4$-buffer pH 6.5, 10× SSC, 0.04% Denhardts and 20% dextran sulphate overnight at 42° with the addition of a PCR-product specific for *CYP*1B1 labeled by random priming [14]. Post-hybridisation washes were performed with 4× SSC and 0.1% SDS at room temperature (rt) for 15 min, with 1× SSC and 0,1% SDS at 40° for 15 min, and with 1× SSC and 0.1% SDS at room temperature for 10 min. Subsequently, the Northern blot was exposed to x-ray film for 3 days.

Immunoblot

The microsome samples were prepared by homogenization of the cells followed by differential ultracentrifugation. The 100,000 *g* fraction was used in TRIS buffer pH 7.4. We loaded 19, 37 and 56 μg microsomal protein into the lanes. As positive control, we used purified *CYP*1B1 (Gentest Corp.) at the concentrations of 3, 6 and 9 pmol. Using 12% polyacrylamidegels with SDS (Novex), blotting was performed onto 0.45 μm cellulose nitrate sheets (Schleicher & Schuell) according to the method of Towbin *et al.* for 1 hr

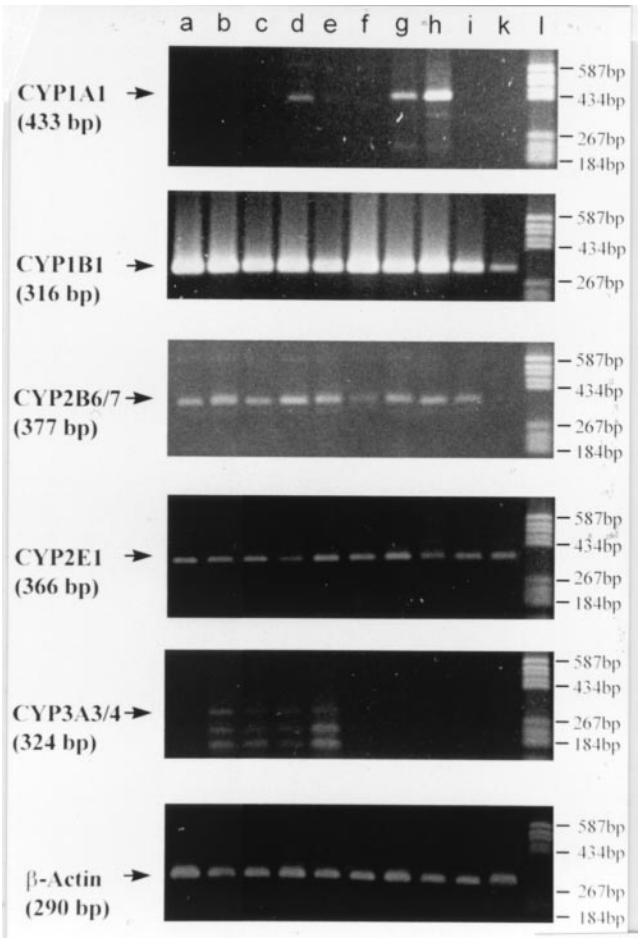

FIG. 1. RT-PCR of control and inducer-treated monocyte/macrophage subsets with primers specific for various P450 isoenzymes and β-actin. (lane a: control; lane b: CSA; lane c: PB; lane d: BA; lane e: ethanol; lane f: dexamethasone; lane g: LPS; lane h: TPA; lane i: subset 27E10; lane k: RM 3/1; lane l: DNA-Marker pBR322 *Hae*III Digest, Sigma).

with 200 volts [15]. Following blocking of the remaining protein binding sites on the membrane with nonfat dry milk powder in PBS, we incubated the blot in the primary antibody solution (#468; 1:1000 anti-CYP1B1 in PBS; see Immunostaining) overnight. After subsequent washing steps, the blot was incubated in the secondary antibody solution (1:1000) anti-rabbit-IgG, phosphatase-labeled (Kirkegaard & Perry) for 4 hr. The blot was developed with a phosphatase staining kit containing nitro-blue tetrazolium and bromo-chloro-indoyl-phosphate for 5 min (Kirkegaard & Perry) to visualize the bands.

Immunostaining

CYP1B1 expression was demonstrated in cytospin preparation of monocytes with an indirect immunoperoxidase technique as described by Zwadlo *et al.* using γ-aminocarbazole (10 min; 37°) as substrate [16]. Anti-CYP1B1 IgG (#468; dilution 1:70) was kindly provided by Üzen Savas (Department of Pharmacology, MSC, Madison, WI) [17–19]. Goat serum (1:10) served as negative control. For additional blocking experiments, the CYP1B1 antibody was preincubated with recombinant human CYP1B1 (0.25 mg protein/mL of diluted antibody) and nonspecific BSA at a similar concentration.

Catalytic Assays

Microsomal fractions were prepared by homogenization of the monocytes followed by differential ultracentrifugation. A radiometric aryl hydrocarbon assay was performed using ^{3}H-benzopyrene as a substrate [20]. The erythromycin *N*-demethylase was measured by the determination of formaldehyde according to Nash [21], as modified by Jugert [22]. The *p*-nitrophenol hydroxylase was determined by the method of Koop [23], using nitrocatechol as the substrate.

RESULTS

The constitutive activity of the aryl hydrocarbon hydroxylase in freshly isolated monocytes was 0.45 ± 0.25 pmol BP metabolites formed/hr/10^6 monocytes. The addition of 20 μM BA for 24 hr increased the activity 6.7-fold to 3.015 pmol BP metabolites formed/hr/10^6 monocytes. CYP3A-mediated erythromycin-demethylase activity was not detectable; however, the p-nitrophenol hydroxylase activity, which is mainly mediated by CYP2E1 was 68.7 ± 4.1 pmol nitrocatechol formed/min/mg of microsomal protein (N = 10) under constitutive conditions.

Expression of CYP1–4 gene families was studied with RT-PCR in human peripheral blood monocytes and macrophages. The results of the RT-PCR studies are displayed in Fig. 1. Figure 2 shows an example of a competitive RT-PCR of CYP1B1 with GAPDH as an internal standard. Percoll-isolated monocytes revealed the expression of CYP1B1, 2E1 and 2B6/7 under constitutive conditions (control). In monocytes purified by adherence or cells

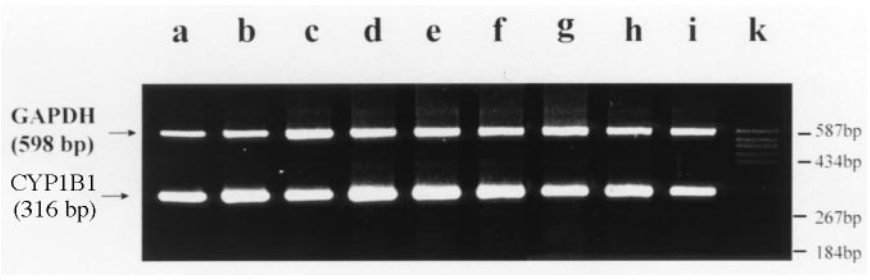

FIG. 2. Competitive RT-PCR of control and inducer-treated monocytes/macrophages with primers specific for CYP1B1 and GAPDH as internal standard. (Lane a: control; lane b: CSA; lane c: PB; lane d: BA; lane e: ethanol; lane f: dexamethasone; lane g: LPS; lane h: TPA; lane i: subset 27E10; lane k: DNA-Marker pBR322 *Hae*III Digest, Sigma).

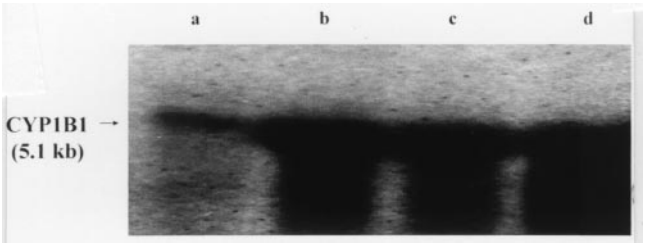

FIG. 3. Northern blot analysis of the *CYP*1B1 expression in blood monocytes. In lane (a) 2 μg of mRNA and in lanes (b–d) 10 μg of total RNA from three different donors were hybridized with a *CYP*1B1 specific probe.

treated for 24 hr with benzanthracene, LPS or TPA, an enhancement in the expression of *CYP*1A1 was observed. Treatment with CSA, PB, BA or ethanol induced the expression of *CYP*3A3/4, which was not detectable without these pretreatments (Fig. 1). The expression of *CYP*1A2 and 3A7 and 4B1 was not found in any specimen (data not shown). However, the strongest RT-PCR signal in monocytes was seen for *CYP*1B1, independent of the stimulation of the cells.

We next studied the expression of *CYP*s in different macrophage subtypes. Subtype 27E10 has been shown to possess pro-inflammatory activity [9, 24], whereas subtype RM3/1 provides anti-inflammatory capabilities [10, 25]. They showed different patterns of *CYP* isoenzyme expression. The pro-inflammatory macrophage subtype 27E10 expressed 1B1, 2E1 and 2B6/7 as shown in Fig. 1. In contrast, the anti-inflammatory macrophage subtype RM 3/1 expressed less 1B1 as shown by PCR (Fig. 1), a comparable amount of 2E1, but no 2B6. The predominant presence of *CYP*1B1 was demonstrated in freshly isolated monocytes by Northern blot (Fig. 3) and immunoblot (Fig. 4), and in monocytes (data not shown) and macrophages

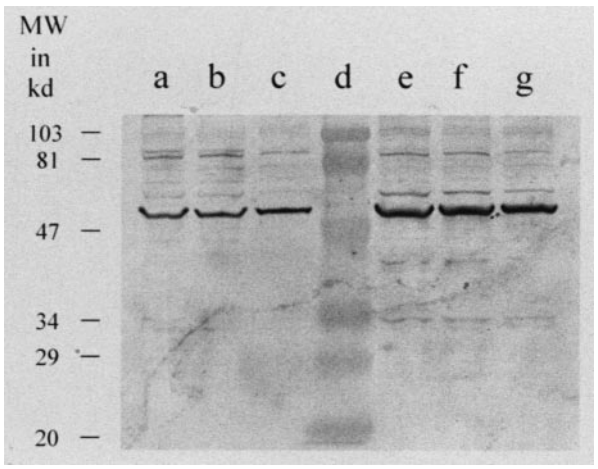

FIG. 4. Immunoblot using an antibody specific for *CYP*1B1. In lane (a–c) microsomal protein from freshly isolated monocytes at different concentrations (56, 37 and 19 μg) was loaded; lane d shows the prestained SDS-PAGE standard (Biorad) with the molecular weights in kilodalton; and lanes (e–g) show the positive control (purified human *CYP* 1B1 with 9, 6 and 3 pmol).

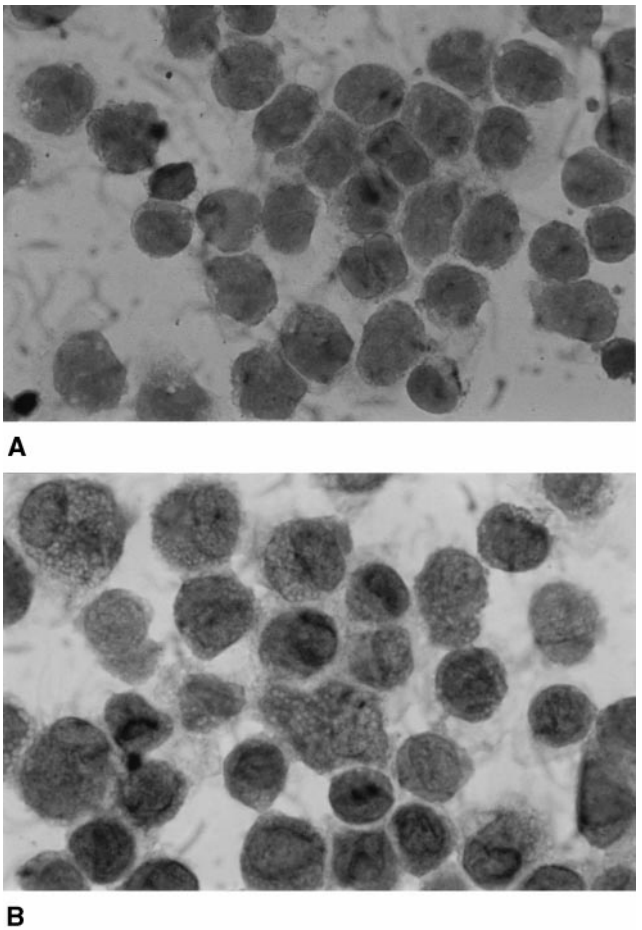

FIG. 5. *CYP*1B1 expression in cytospin preparation of macrophages (day 4) using indirect immunoperoxidase technique. (A) Control with antibody blocked by recombinant *CYP*1B1 protein (see Methods). (B) Red staining indicates *CYP*1B1 immunoreactivity.

(day 4) by immunohistochemical staining with *CYP*1B1-specific antibodies (Fig. 5). Blocking experiments (Fig. 5) showed that preincubation of the antibody with excess recombinant human *CYP*1B1 prevented staining (Fig. 5A), while preincubation with a nonspecific protein (BSA) at similar concentration did not alter staining (Fig. 5B).

DISCUSSION

The present study demonstrates that human blood monocytes and monocyte-derived macrophages constitutively express P450 isoenzymes. Of particular interest is the strong and constitutive expression of *CYP*1B1 in monocytes and macrophages which was demonstrated both on the mRNA and protein levels. *CYP*1B1 is the only gene of the *CYP*1B gene family which has been mapped to human chromosome 2p21–22, which contains three exons and two introns, and is present especially in extrahepatic tissues [26, 27]. The mRNA expression of *CYP*1A1 is inducible by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin in human tissues [26]. Furthermore, there are several studies showing that the expression of the mRNA of this enzyme compared to *CYP*1A1 is

different in several different tissues [28–30]. Our observation that this enzyme is the main *CYP* in human monocytes and monocyte-derived macrophages under constitutive conditions and that it is not induced by BA, which binds to the Ah-receptor (similar to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin), suggests another regulation of this mRNA expression in these cells.

*CYP*1A1 and 3A3/4 enzymes were induced by pretreatment with BA or CSA, and PB respectively. In addition, *CYP*1A1 expression was induced by chemicals or xenobiotics known to activate monocytes (e.g. TPA or LPS), but also by plastic adherence, a process which is known to alter the differentiation of monocytes [31]. On the other hand, *CYP*1B1 expression was found to be rather clearly independent of the isolation procedures of monocytes on the activation stage. In previous studies, it has been observed that the expression pattern of P450 isoenzymes in cells, including hepatocytes and keratinocytes, depends on the level of differentiation [32]. For that reason, we examined monocytes undergoing differentiation to mature macrophages directly after isolation and cultivation for 2, 4 and 7 days, but no change in the P450 mRNA expression levels could be revealed. We were able to detect this *CYP*1B1 by using immunohistochemical staining of monocytes (Fig. 5) without their pretreatment with inducing agents. This technique was recommended especially for extrahepatic tissues [33]. Recently performed immunohistochemical staining suggested that *CYP*1B1 is present in a wide range of malignant tumors [30].

The macrophage subtype RM 3/1 contains fewer *CYP* isoenzymes than the 27 E10 subtype. The RM 3/1 subset is associated with the antiinflammatory functions because it appears during down-regulation in inflammatory processes and secretes anti-inflammatory proteins [10, 25]. The 27E10 subset represents a pro-inflammatory, activated type of macrophage found only in acute inflamed tissues and producing large amounts of inflammatory mediators such as prostaglandin E_2, tumor necrosis factor (TNF) and interleukin 1 [9, 24]. The stronger expression of P450 isoenzymes in 27E10 macrophages than in RM 3/1 cells may be linked to the activation stage of this subset, e.g. antigen processing. Our results suggest that cell differentiation might have a major influence on P450 expression in these cells, although under all conditions so far investigated *CYP*1B1 is still the major isoenzyme of this gene family found to be expressed in monocytes.

The inducibility of P450 isoenzymes is one mechanism by which small molecular weight compounds can influence the role of these enzymes in their own toxic reactions [34]. *CYP*1A1 and *CYP*2E1 mRNA as well as p-nitrophenol-hydroxylase activity are present in monocytes after pretreatment with known inducers of P450 isoenzymes, which is of interest because these isoenzymes underlie a genetic polymorphism in the population [35, 36]. Whether this contributes to individuals having a different risk of developing allergic reactions to small molecular weight compounds will be a matter of subsequent studies. The influence of the likelihood of such a genetic polymorphism of xenobiotic-metabolising enzymes has been shown at least for the acetyltransferase in the case of sulphonamide- and p-phenylendiamine sensitization [37, 38, 39].

The knowledge concerning antigen processing of small molecular weight antigens is still in its infancy, especially if one compares it with our knowledge as to the processing of high molecular weight compounds [40]. It is not clear whether the small molecular weight antigen binds to a protein outside of the antigen-presenting cell, which is then taken up by these cells in order to be processed, or if it is absorbed by antigen-presenting cells in order to be bound to a protein and subsequently processed by the cells or finally if it binds directly to, at least, the MHCI[define] complex from the outside to stimulate at least CD8+ T lymphocytes [40, 41]. There are several techniques now available to study the antigenic potency, for example, of contact allergens, and transfected cell lines to study the metabolic capacity of specific P450 isoenzymes. However, an essential prerequisite to use such systems to study the role of this metabolism in allergic reactions mediated by small molecular weight compounds are studies such as this providing us with more details concerning P450 isoenzymes present in antigen-presenting cells. Recently, the human *CYP*1B1 cDNA was expressed in Saccharomyces cerevisiae, and several substrates, including several aryl amines, were characterized [42]. Therefore, it might be most interesting that at least in monocytes and monocyte-derived macrophages *CYP*1B1 is the major P450 isoenzyme under constitutive conditions as well as after pretreatment with several known P450 inducers or by immunologic induction of these cells.

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