Research Article

Myeloid Human Cell Lines Lack Functional Regulation of Aryl Hydrocarbon Receptor-Dependent Phase I Genes

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Summary

Primary dendritic cells and myeloid cell lines are used to assess the skin sensitization hazard in *in vitro* approaches. The aryl hydrocarbon receptor (AhR) modulates expression of CYP enzymes, which play a significant role in the bioactivation of various xenobiotics. These studies revealed a strong constitutive expression of the AhR in primary human monocytes, monocyte-derived immature dendritic cells (iDC) and cord blood-derived Langerhans cells (LC). In contrast, mRNA and protein expression of AhR was hardly detectable in the cell lines THP-1 and MUTZ-3. U937 cells and MUTZ-3-derived dendritic (MUTZ-DC) or Langerhans cells (MUTZ-LC) showed about half the expression of AhR compared to iDC. Incubation of cells with the specific AhR-inducer benzo[a]anthracene resulted in an upregulation of CYP and IL-1β mRNA expression in primary monocytes and iDC. CYP1A1 but not CYP1B1 and IL-1β expression was increased by benzo[a]anthracene in these cell lines except for U937 cells. AhR-independent CYP genes were not regulated by benzo[a]anthracene. Constitutive mRNA expression of other non AhR-dependent CYP enzymes was higher in some of the cell lines compared to the corresponding primary cells. This study demonstrates significant differences in expression and regulation of phase I genes in cell lines currently used for *in vitro* skin sensitization hazard assessment compared to primary cells. Additional studies are required regarding the combination of cutaneous xenobiotic metabolizing enzymes and APC-sensitization for the development of valid *in vitro* models for skin sensitization assessment.

Keywords: AhR, CYP enzymes, dendritic cells, skin sensitization

1 Introduction

Cytochrome P450 enzymes (CYP) represent a key metabolic enzyme family capable of metabolizing drugs and chemicals in hepatic and extrahepatic tissues including the skin. The induction of CYP1A1 and CYP1B1 enhances the metabolism of polycyclic aromatic hydrocarbons (PAH), particularly benzo[a]pyrene and benzo[a]anthracene (Modi et al., 2012), which are potent carcinogens and are also able to induce contact hypersensitivity (Anderson et al., 1995). Humans are exposed to PAHs and related chemicals mainly through tobacco smoking and automobile exhaust (Finlayson-Pitts and Pitts, 1997; Rubin, 2001).

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor with multiple functions in adaptive metabolism (Bock and Köhle, 2009). AhR in its dormant state is located in association with a complex of HSP90 (heat-shock protein 90), AIP (AhR interacting protein) and HSP90 co-chaperone p23 in the cytoplasm (Chen and Perdew, 1994). Upon ligand binding, AhR is activated by a conformational change

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Abbreviations

AhR, aryl hydrocarbon receptor; ARNT, AhR nuclear translocator; CYP, cytochrome P450 enzymes; GST, glutathione S-0transferase; HSP90, heat-shock protein 90; LC, Langerhans cells; moDC, monocyte-derived dendritic cells; MRP; multidrug resistance-associated protein; Nrf2; nuclear factor (erythroid-derived 2)-like 2; NQO1, NAD(P)H dehydrogenase, quinone 1; PAH, polycyclic aromatic hydrocarbons; UGT, UDP glucuronosyltransferase; XRE, xenobiotic response element

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that exposes a nuclear localization signal (Ikuta et al., 1998). HSP90 is released from the complex and the receptor translocates to the nucleus, where it forms a heterodimer with AhR nuclear translocator (ARNT) and binds to xenobiotic response element (XRE) (Whitelaw et al., 1993). XREs are found in the promotor regions of several genes involved in the metabolism of xenobiotics, including the CYP enzymes CYP1A1, CYP1A2 and CYP1B1. The AhR pathway is regulated by the AhR repressor (AhRR), a target gene of the AhR in a negative feedback loop (Ma, 2001; Esser et al., 2009; Jux et al., 2009). Liver and lung exhibit high levels of AhR expression (Dolwick et al., 1993; Hayashi et al., 1994). However, it was found that some specific hematopoietic stem cells, dendritic cells, particular subsets of thymocytes and T-cells have similar or even higher levels of AhR expression than the liver (Esser et al., 2009; Frericks et al., 2007; Hirabayashi and Inoue, 2009; Veldhoen et al., 2009).

In previous studies we were able to show that normal human epidermal keratinocytes (Baron et al., 2001, 2008) and also human skin equivalents (Neis et al., 2010) express a specific profile of CYP enzymes. In addition, we found that various CYP enzymes are expressed in dendritic cells (Sieben et al., 1999) as well as in monocytes and macrophages (Baron et al., 1998). Furthermore, we demonstrated using monocyte-derived dendritic cells (moDC) and THP-1 cells that CYPs play a significant role in the activation of prohaptens to highly reactive species which bind to proteins and become the nominative antigen (Bergström et al., 2007; Merk et al., 2007; Ott et al., 2009). The αβ-unsaturated oxime R-carvoxime is bioactivated by human cutaneous CYP1A1, CYP1B1 and, to some extent, by CYP2B6, thus forming highly allergenic metabolites, and has the potential to induce its own bioactivation pathway (induces CYP1B1), particularly in antigen-presenting cells (Ott et al., 2009).

The establishment of *in vitro* sensitization methods for the screening of new chemicals is of major importance for the reduction of animal testing. One approach to quantify the sensitization potential of a chemical *in vitro* is to determine dendritic cell (DC) activation. Low molecular weight chemicals induce the activation of DCs, which leads to upregulation of surface marker expression, cytokine production and Nrf2-dependent antioxidant gene products. Human cell lines including THP-1, MUTZ-3 and U937 should undergo similar alterations after activation, which indicate their potential to behave like a DC. In addition to primary DCs, these cell lines are used as an alternative tool to predict skin sensitization (Basketter et al., 2008; Ott et al., 2010; Python et al., 2009).

Here, we investigated the expression of AhR and ARNT in the human cell lines THP-1, U937 and MUTZ-3 in comparison to primary human monocytes, immature moDC (iDC) and cord blood-derived Langerhans cells (LC) using real-time PCR analysis and immunohistochemistry. Furthermore, we analyzed the gene expression profile of AhR-dependent and -independent CYP450 enzymes and of IL-1β in cells treated with benzo[a]anthracene and unstimulated control cells by real-time PCR analysis.

2 Materials and methods

2.1 *In vitro* culture

All cell culture materials used were non-cytotoxic, non-pyrogenic tested to less than 0.1 EU/ml by the companies they were purchased from.

Monocyte-derived dendritic cells

Human peripheral blood mononuclear cells (PBMCs) from a total of six healthy donors were separated from single buffy coats (Department of Transfusion Medicine, University Hospital Aachen, Germany) over a Ficoll-Paque gradient (Amersham Pharmacia Biotech, Uppsala, Sweden). Due to restrictions of the Aachen ethics committee only anonymized probes may be used for cell culture experiments.

CD3, CD7, CD16, CD19, CD56, CD123 and Glycophorin A positive leukocytes were depleted using a negative monocyte isolation kit (human Monocyte Isolation Kit II, Miltenyi Biotec, Bergisch Gladbach, Germany). The isolated, unlabeled monocytes were suspended in medium consisting of RPMI 1640 + GlutaMAX™-I (Invitrogen, Darmstadt, Germany) enriched with 3% heat-inactivated autologous plasma. The cell suspension was plated at a density of $3x10^6$ cells per reaction well in a 6-well plate. In order to induce DC differentiation, the culture medium was supplemented with 800 U/ml GM-CSF (R&D-Systems, Bühlmann, Basel, Switzerland, 215-GM-050/CF) and 1000 U/ml IL-4 (R&D-Systems, Wiesbaden, Germany, 204-IL-050/CF) and cells were kept at 37°C and 5% CO_2 for 6 days. On days 2, 4 and 6 the culture medium was refreshed and 1600 U/ml GM-CSF and 1000 U/ml IL-4 were added. On day 5 the resulting DC phenotype was determined by flow cytometric analysis. At this time point, the cells displayed a phenotype characteristic for immature DC, i.e., $CD1a^{high}$, $CD80^{intermediate}$, $CD86^{low}$, $CD83^{negative}$, $CD14^{negative}$. On day 6 suspension cells were transferred to new 6-well-plates and stimulated with benzo[a]anthracene (Sigma-Aldrich, Munich, Germany).

Langerhans cells (LC)

Cord blood-derived $CD34^+$ progenitor cells were purchased from STEMCELL Technologies, Grenoble, France. To induce LC differentiation, cells were transferred into T-150 flasks and cultured in 30 ml RPMI medium supplemented with 10% FCS (Biochrom, Berlin, Germany), 100 ng/ml GM-CSF, 2.5 ng/ml TNF-α (R&D-Systems, Wiesbaden, Germany, 204-IL-050/CF) and 25 ng/ml SCF (R&D Systems, Wiesbaden, Germany, 255-SC-050/CF). On days 2-4 culture medium was refreshed. On day 5, medium was changed to RPMI1640 + 10% FCS + 1 ng/ml TGF-β1 (R&D Systems, Wiesbaden, Germany, 240-B-010/CF). Medium was refreshed on day 7 and on days 9-10. On day 12, LC-phenotype was determined by flow cytometric analysis. Cells displayed a phenotype characteristic for LCs, i.e., $CD207^{high}$ and $CD1a^{high}$. Cells were stimulated with benzo[a]anthracene on day 12.

MUTZ-3

MUTZ-3, an acute myelogenous leukemia cell line, was purchased from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany, ACC295) and was cultured

in Minimum Essential Medium alpha (MEM-α) + GlutaMAX™ (Gibco/Invitrogen, Darmstadt, Germany) supplemented with ribonucleosides and deoxyribonucleosides, 20% FCS (Biochrom, Berlin, Germany), 2 mM L-Glutamine (Life Technologies, Darmstadt, Germany), 50 μM β-mercaptoethanol (Sigma-Aldrich, Munich, Germany) and 10% of conditioned medium from the renal cell carcinoma cell line 5637 at 37°C in a 5% CO_2 humidified incubator. Cells were subcultured at a split ratio of 1:2 twice per week. Cells were stimulated with benzo[a]anthracene on days 6-7.

5637, a renal cell carcinoma cell line, was purchased from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany, ACC35) and was cultured in RPMI medium supplemented with 10% FCS. The cells were subcultured at a split ratio of 1:4 to 1:5 and passaged every 3-4 days.

MUTZ-DC

Immature MUTZ-DCs (MUTZ-derived dendritic cells) were generated from MUTZ-3 progenitor cells. MUTZ-3 cells were cultured at a density of $2x10^5$ cells/ml in 12-well plates for 6-7 days in Minimum Essential Medium alpha (MEM-α) + GlutaMAX™ (Gibco/Invitrogen, Darmstadt, Germany) supplemented with ribonucleosides and deoxyribonucleosides, 20% FCS (Biochrom, Berlin, Germany), 2 mM L-Glutamine (Life Technologies, Darmstadt, Germany), 50 μM β-mercaptoethanol (Sigma-Aldrich, Munich, Germany) without conditioned medium of 5637 cells supplemented with 100 ng/ml GM-CSF, 20 ng/ml IL-4 and 2.5 ng/ml TNF-α. On day 3, fresh cytokines equivalent to 1 ml of medium were added to each well. Cells were stimulated with benzo[a]anthracene at day 6-7.

MUTZ-LC

Immature MUTZ-LCs (MUTZ-derived Langerhans Cells) were generated from MUTZ-3 progenitor cells. MUTZ-3 cells were cultured at a density of $1x10^5$ cells/ml in 12-well plates for 9 days in Minimum Essential Medium alpha (MEM-α) + GlutaMAX™ (Gibco/Invitrogen, Darmstadt, Germany) supplemented with ribonucleosides and deoxyribonucleosides, 20% FCS (Biochrom, Berlin, Germany), 2 mM L-Glutamine (Life Technologies, Darmstadt, Germany), 50 μM β-mercaptoethanol (Sigma-Aldrich, Munich, Germany) without conditioned medium of 5637 cells supplemented with 100 ng/ml GM-CSF, 10 ng/ml TGF-β and 2.5 ng/ml TNF-α (R&D Systems, Wiesbaden, Germany). On day 3 and 6, cytokines equivalent to 1 ml of medium were added to each well. Cells were stimulated with benzo[a]anthracene at day 9.

THP-1

Human THP-1 monocytic-like cells were obtained from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany) and cultured as suspension cells in RPMI-1640 (Gibco/Invitrogen, Darmstadt, Germany) supplemented with 10% FCS (Biochrom, Berlin, Germany) at 37°C in a 5% CO_2 humidified incubator. The cells were subcultured at a split ratio of 1:4 to 1:6 and passaged every 3-4 days. Cells were transferred to new 6-well-plates and stimulated with benzo[a] anthracene.

U937

Human U937 cell line was purchased from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany, ACC5) and cultured as suspension cells in RPMI 1640 (Gibco/Invitrogen, Darmstadt, Germany) supplemented with 10% FCS (Biochrom, Berlin, Germany) at 37°C and 5% CO_2. The cells were subcultured at a split ratio of 1:5 to 1:10 and passaged every 3-4 days. Cells were transferred to new 6-well-plates and stimulated with benzo[a]anthracene

Cell lines were regularly tested for mycoplasma contamination and were used at limited passage numbers (MUTZ-3 (including MUTZ-LC and MUTZ-DC) passage 17, THP-1 passage 9, U937 passage 8).

2.2 Cell stimulation with benzo[a]anthracene

A stock solution of benzo[a]anthracene ($C_{18}H_{12}$, IUPAC Tetraphene, MW 228.29, CAS number 56-55-3; Sigma-Aldrich, Munich, Germany) was prepared in DMSO at a concentration of 10^{-2} M. Primary cells and myeloid cell lines were stimulated with a stock solution of benzo[a]anthracene prediluted in cell specific medium as described above, at a concentration of 10^{-6} M for 24 h.

2.3 RNA isolation

Total RNA was isolated using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, including on-column digestion of DNA with RNase-free DNase I. The RNA was quantified by photometric measurement (NanoDrop Technologies, Wilmington, DE, USA).

2.4 Quantitative Real-Time Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

Purified RNA was reverse transcribed with the High Capacity RNA-to-cDNA Master Mix (Applied Biosystems, Darmstadt, Germany) according to the manufacturer's instructions. Taq Man experiments were carried out on an ABI Prism 7000 sequence detection system (Applied Biosystems, Darmstadt, Germany) using Assays-on-Demand gene expression products for AhR (Hs00169233_m1), ARNT (Hs00231048_m1), CYP1A1 (Hs00153120_m1), CYP1B1 (Hs00164383_m1), IL1ß (Hs00174097_m1), CYP2S1 (Hs00998125_m1), CYP2E1 (Hs00c559367_m1) and CYP3A5 (Hs00241417_m1) according to the manufacturer's recommendations. An Assay-on-Demand product for cyclophilin A (Hs99999904_m1) was used as an internal reference to normalize the target transcripts. Results were analyzed with the 7300 System SDS Software (Applied Biosystems, Darmstadt, Germany). All measurements were performed in triplicates in separate reaction wells.

2.5 Immunocytological analysis of AhR expression

Cytospin preparations of moDCs, LCs, MUTZ-3, THP-1 and U937 (~ 0.1 x 10^6 cells) were stained with monoclonal antibodies against CYP1A1 (ab3568), CYP1B1 (ab33586) and AhR (ab2770) (Abcam, Cambridge, UK) each for 1 h and protein expression was detected using a labeled streptavidin-biotin

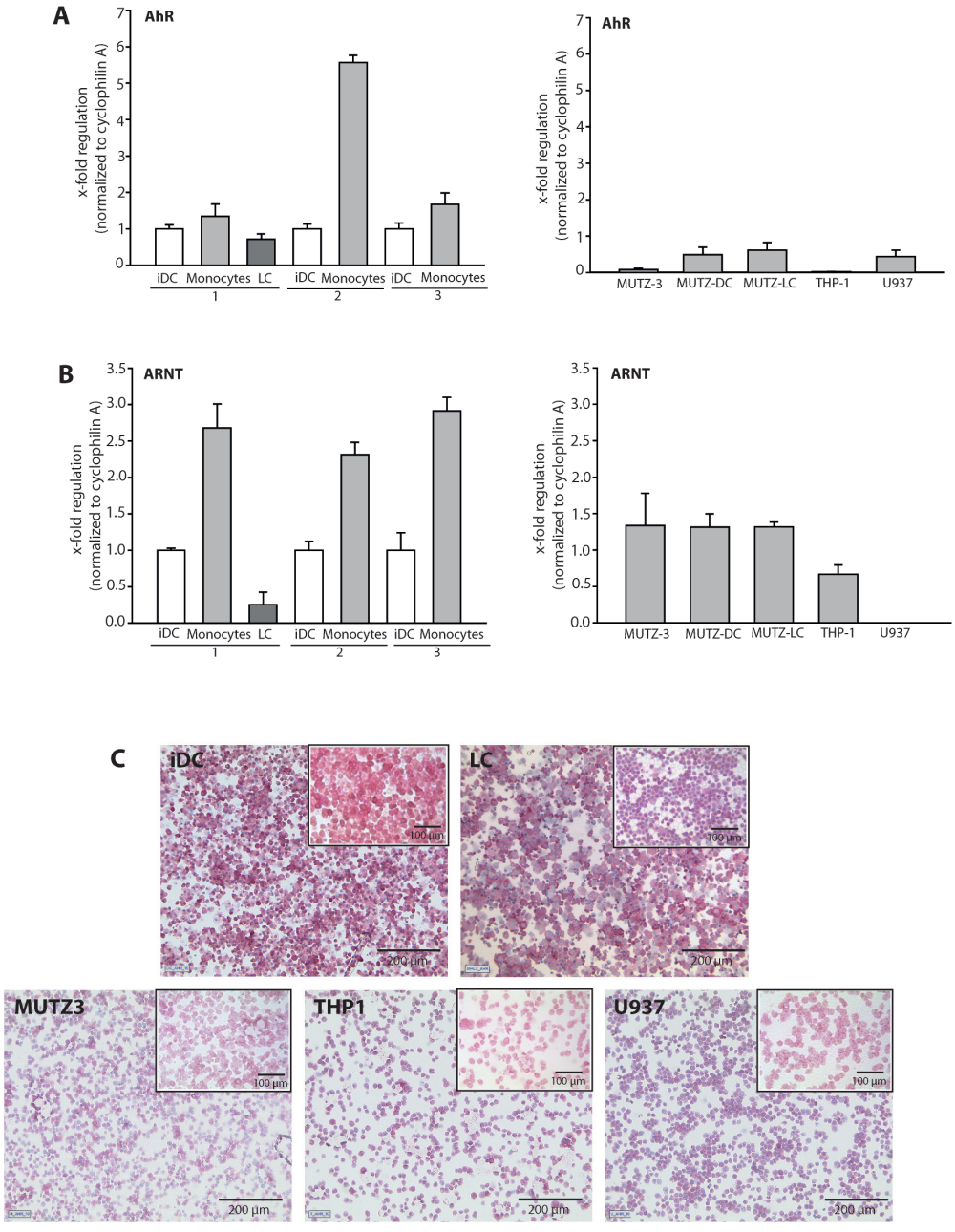

Fig. 1: Expression of AhR and ARNT in monocytes, iDC and LC and in the myeloid cell lines MUTZ-3, MUTZ-DC, MUTZ-LC, THP-1 and U937

mRNA expression of AhR A) and ARNT B) was measured by TaqMan real-time PCR analysis. The relative RNA levels are presented as x-fold regulation compared to immature monocyte-derived dendritic cells (iDC) (= 1) and were normalized against cyclophilin A. Data of three blood donors are displayed for primary cells except for LC. Mean values and standard deviation of three biological replicates are displayed for the myeloid cell lines. C) Immunocytological representative analysis of AhR protein expression. Cytospin preparations of iDC, LC, MUTZ-3, THP-1 and U937 cells were stained with a monoclonal antibody against AhR and protein expression was detected using the Dako REAL Detection System. Counterstaining was performed using haematoxilin. Magnification x10, framed regions are separately enlarged (x20)

method according to the manufacturer's instructions ("Dako REAL™ Detection System, Alkaline Phosphatase/RED, Rabbit/Mouse" kit, Dako, Hamburg, Germany). Counterstaining was performed with hematoxylin (Sigma Aldrich, Munich, Germany). Negative controls were stained with isotype mouse IgG1 (Dako; Hamburg, Germany) (see supplementary file at http://dx.doi.org/10.14573/altex.1502041s).

2.6 Statistical analysis

Data of primary cells are displayed as single experiments of 3 biological replicates except for LC. Only one LC experiment was performed exemplarily. Data of 3 biological replicates of the cell lines are given as arithmetical means +/- standard deviation. A paired t-test was performed to test for statistical significance between unstimulated cells and benzanthracene-stimulated cells with respect to AhR-dependent genes. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

3 Results

We measured AhR and ARNT expression as well as the expression of AhR-dependent genes and additional CYP enzymes to compare the metabolizing capacity of primary dendritic cells (iDC) with the cell lines THP-1, MUTZ-3, MUTZ-DC, MUTZ-LC and U937 used in *in vitro* assays for the detection of skin sensitization.

Figure 1 displays the relation of AhR expression in primary dendritic cells (iDC), monocytes, Langerhans cells (LC) and the cell lines THP-1, MUTZ-3, MUTZ-DC, MUTZ-LC and U937. Monocytes express the highest level of AhR (Fig. 1A) while the expression of AhR in iDC and in the single LC sample was comparable (Fig. 1A,C). Although there was an interindividual donor-dependent variance in gene expression of primary cells, the tendency of the observed gene regulation was similar. Regarding the cell lines, MUTZ-3 and THP-1 cells showed a lower expression of AhR compared to iDC and monocytes (Fig. 1A). This result was also confirmed on protein level by immunostaining (Fig. 1C). MUTZ-DC, MUTZ-LC and U937 cells express about half the amount of AhR compared to iDC (Fig. 1A,C).

ARNT, the AhR nuclear translocator, is expressed most prominently by monocytes followed by similar levels in iDC, MUTZ-3, MUTZ-DC and MUTZ-LC (Fig. 1B). The ARNT expression in the single LC sample and THP-1 cells was lower and in U937 cells ARNT expression was not detectable (Fig. 1B).

To measure the expression of AhR-dependent genes, the different cells were stimulated with the AhR-ligand benzo[a]anthracene for 24 h. The expression of CYP1A1, CYP1B1 and IL-1β was increased in all samples of iDC, monocytes and LC by benzo[a]anthracene in comparison to unstimulated controls (Fig. 2A,C,E). MUTZ-3, MUTZ-DC, MUTZ-LC and THP-1 cells also showed a statistically significantly increased expression of the AhR target gene CYP1A1 after incubation with benzo[a] anthracene (Fig. 2B). CYP1B1 upregulation by benzo[a]anthracene could be confirmed as statistically significant for MUTZ-LC and THP-1 (Fig. 2D). However, CYP1B1 expression in unstimulated cells or cells treated with benzo[a]anthracene was significantly lower compared to iDC and monocytes (Fig. 2D). U937 cells did not show an increased expression of either CYP1A1 or CYP1B1 after stimulation with benzo[a]anthracene. IL-1β is strongly expressed by LC, MUTZ-DC and MUTZ-LC compared to iDC (Fig. 2E, F). In contrast to the primary cells, no increased expression of IL-1β induced by benzo[a]anthracene in the myeloid cell lines was detectable (Fig. 2F).

We further investigated the gene expression of AhR-independent CYP isoenzymes in iDC, monocytes and LC as well as in the cell lines THP-1, MUTZ-3, MUTZ-DC, MUTZ-LC and U937, both in unstimulated cells and after incubation with the AhR-ligand benzo[a]anthracene for 24 h. Analysis of the expression of the CYP enzymes CYP2S1, CYP2E1 and CYP3A5 revealed no regulation by benzo[a]anthracene in all cell types analyzed except for a slight upregulation of CYP2S1 in iDC (Fig. 3A), indicating that these CYP enzymes are no target genes of AhR (Fig. 3A-F). Figure 3A and B show that the cell lines THP-1, MUTZ-3 and U937 express less CYP2S1 than iDC, monocytes and LC. On the other hand, CYP2S1 is approximately 2.5 and 4.4 fold expressed in MUTZ-DC and MUTZ-LC, respectively, compared to iDC (Fig. 3B). Monocytes, LC, MUTZ-DC, MUTZ-LC and THP-1 cells show a trend towards a slightly higher expression of CYP2E1 compared to iDC (Fig. 3C,D). In contrast, CYP2E1 is strongly expressed in the myeloid cell lines MUTZ-3 and U937 (Fig. 3D). LC express a high amount of CYP3A5 compared to iDC (Fig. 3E). A strong expression of CYP3A5 is also detectable in MUTZ-3, MUTZ-DC and MUTZ-LC compared to iDC. In contrast, CYP3A5 expression is hardly detectable in monocytes, iDC, THP-1 and U937 (Fig. 3E, F).

4 Discussion

There is an urgent need for alternative *in vitro* test systems for assessment of skin sensitizing chemicals. Human primary dendritic cells as well as myeloid cell lines are used in *in vitro* approaches to predict skin sensitization (Basketter et al., 2008; Ott et al., 2010; Python et al., 2009), e.g., the Myeloid U937 Skin Sensitization Test (MUSST), which measures increase of CD86 expression (Ade et al., 2006; Python et al., 2007), the human Cell Line Activation Test (h-CLAT), which assesses increase of CD86 and combined CD54 expression (Sakaguchi et al., 2006), or the Genomic Allergen Rapid Detection (GARD), which is based on a signature of predictive genes differentially regulated in the MUTZ3 cell line when stimulated with sensitizing compared to non-sensitizing compounds (Johansson et al., 2013).

The current study focused on the comparison of the metabolizing capacity of human primary dendritic cells with the cell lines THP-1, MUTZ-3, MUTZ-DC, MUTZ-LC and U937. We investigated the expression of AhR and its cofactor ARNT and measured the mRNA levels of AhR-dependent genes (CYP1A1, CYP1B1, IL-1β) and of AhR-independent metabolizing CYP enzymes (CYP2S1, CYP2E1, CYP3A5) in unstimulated cells and cells stimulated with the AhR-ligand benzo[a]anthracene.

Our data show that monocytes have the highest expression of AhR followed by iDC, LC, MUTZ-LC, MUTZ-DC and U937.

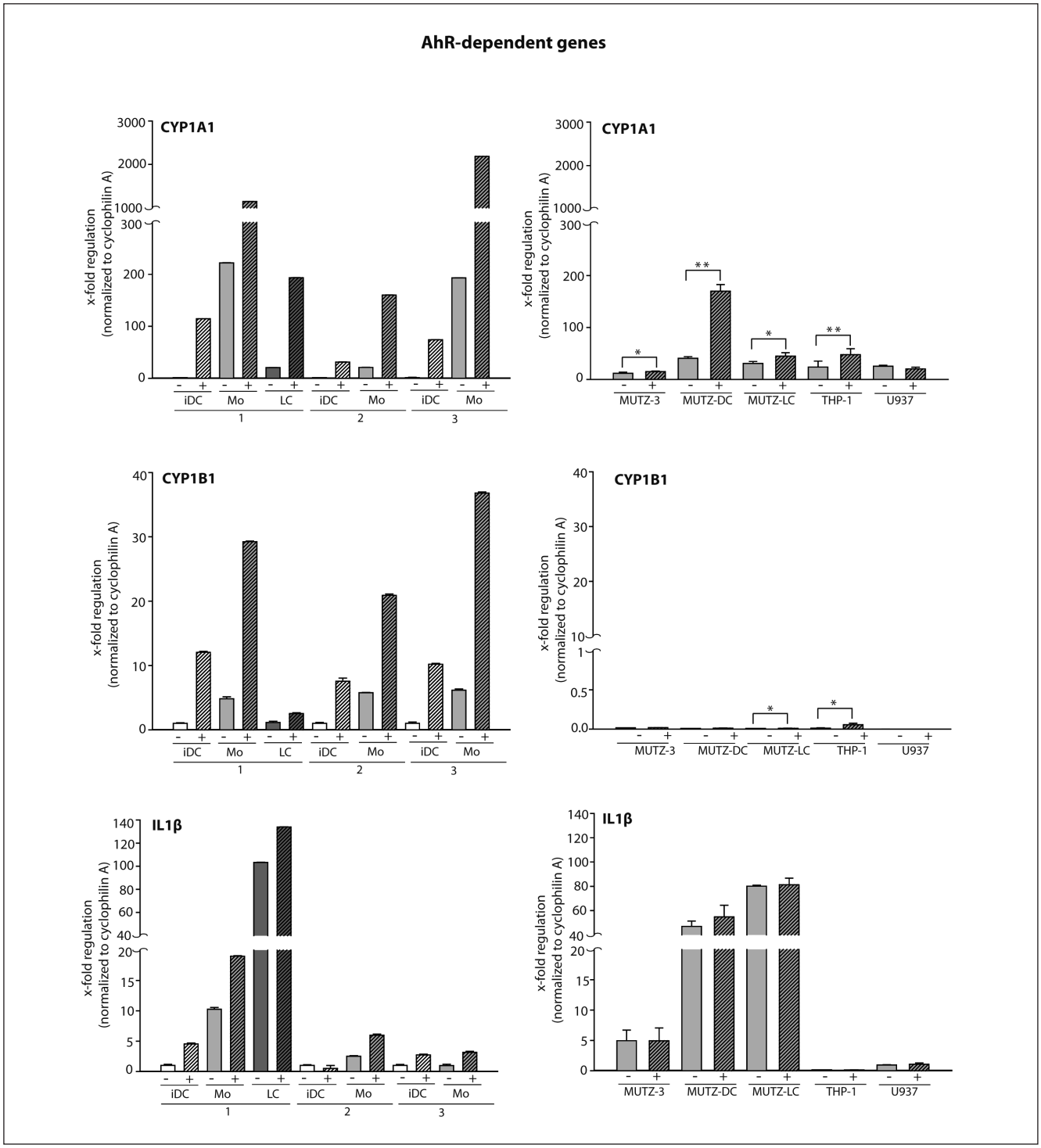

Fig. 2: Benzo[a]anthracene induced mRNA expression of AhR-dependent genes in monocytes, iDC and LC and in the myeloid cell lines MUTZ-3, MUTZ-DC, MUTZ-LC, THP-1 and U937

mRNA expression of CYP1A1 (A/B), CYP1B1 (C/D) and IL-1β (E/F) was measured by TaqMan real-time PCR analysis in unstimulated cells and after stimulation with 10^{-6} M benzo[a]anthracene for 24 h. Measurement was performed in triplicates. Data of primary cells from three single blood donors is displayed except for LC (one donor). Data of myeloid cell lines is presented as mean value ± standard deviation. The relative RNA levels of the primary cells are displayed as x-fold regulation compared to unstimulated immature monocyte-derived dendritic cells (iDC) (= 1), whereas RNA levels of the myeloid cell lines are displayed as x-fold regulation compared to the mean value of iDC from three blood donors (= 1). RNA levels were normalized against cyclophilin A. A paired t-test was performed to test for statistical significance between unstimulated cells and benzo[a]anthracene-stimulated cells. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

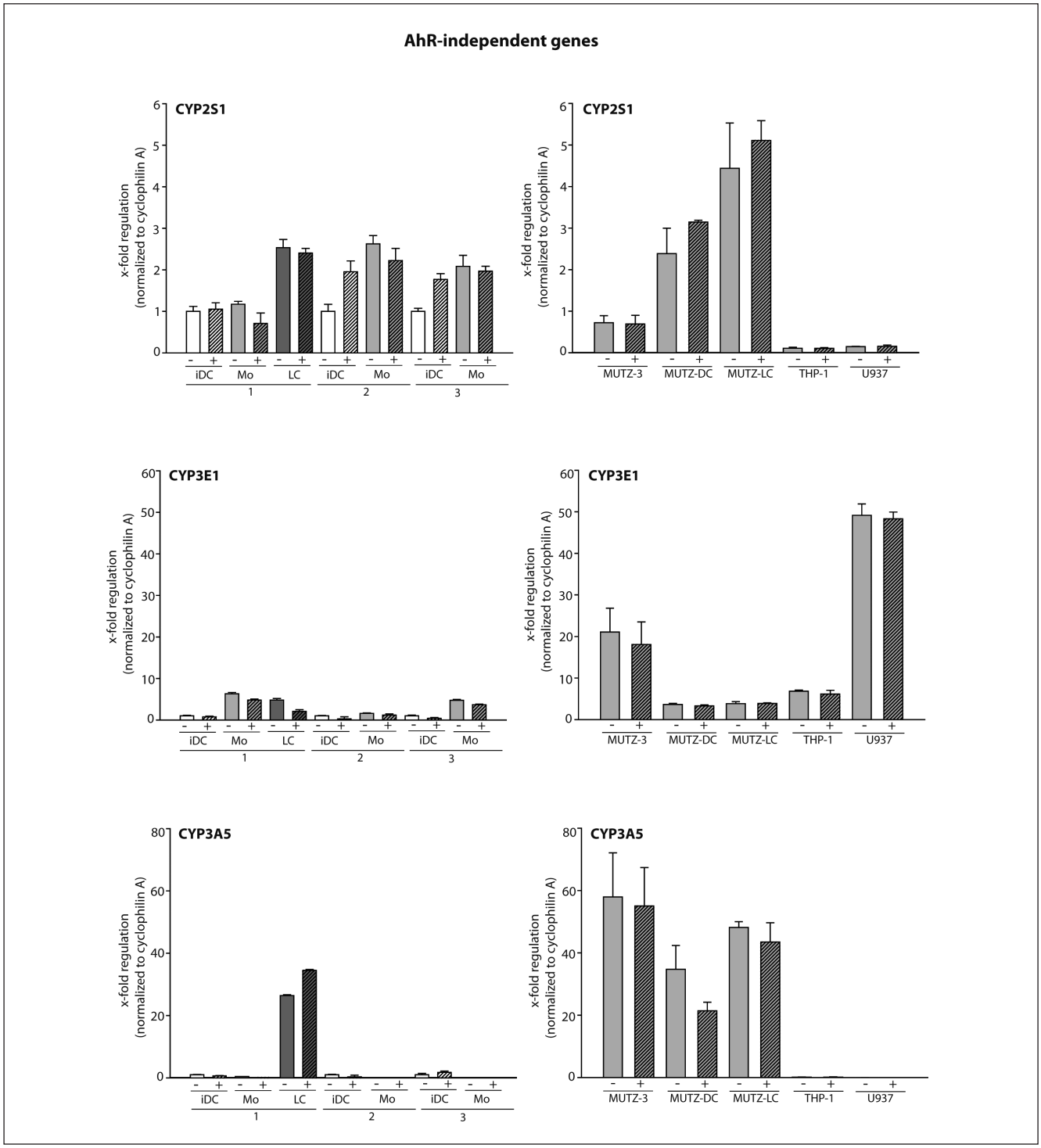

Fig. 3: Benzo[a]anthracene induced mRNA expression of AhR-independent genes in monocytes, iDC and LC and in the myeloid cell lines MUTZ-3, MUTZ-DC, MUTZ-LC, THP-1 and U937

mRNA expression of CYP2S1 (A/B), CYP2E1 (C/D) and CYP3A5 (E/F) was measured by TaqMan real-time PCR analysis in unstimulated cells and after stimulation with 10^{-6} M benzo[a]anthracene for 24 h. Measurement was performed in triplicates. Data of primary cells from three single blood donors is displayed except for LC. Data of myeloid cell lines is presented as mean value ± standard deviation. The relative RNA levels of the primary cells are displayed as x-fold regulation compared to unstimulated immature monocyte-derived dendritic cells (iDC) (= 1), whereas RNA levels of the myeloid cell lines are displayed as x-fold regulation compared to the mean value of iDC from three blood donors (= 1). RNA levels were normalized against cyclophilin A.

AhR expression in MUTZ-3 and THP-1 was very low. Regarding the expression of ARNT, again the expression in monocytes was highest, followed by iDC, MUTZ-3, MUTZ-DC, MUTZ-LC, LC, THP-1 and no detectable levels in U937.

Target gene expression induced by AhR is dependent on ARNT. ARNT is expressed in many tissues and can dimerize with a number of bHLH/PAS (basic helix-loop-helix/Per-ARNT-Sim) proteins besides AhR, suggesting that ARNT functions in a broad range of physiological responses (Ma, 2001). Although AhR is expressed at very low levels in THP-1 and MUTZ-3 cells, an increased expression of CYP1A1 could be observed after induction with benzo[a]anthracene albeit to a lesser extent than in iDC and monocytes. However, CYP1B1 and IL-1β expression was not upregulated by benzo[a]anthracene on a biologically relevant level.

Treatment of THP-1 cells with prohaptens in the presence or absence of arochlor-induced rat liver S9 homogenate suggested that endogenous CYP activity in the THP-1 cells is insufficient for prohapten screening and might result in false negative findings for prohaptens (Chipinda et al., 2011). Recently, new biomarkers for chemical sensitization have been presented, including the surface molecules PD-L1 and DCIR and the soluble factors MIP-1α, NAP-2 and IL-16 (Hitzler et al., 2013). The authors showed that monocyte-derived dendritic cells (moDCs) revealed higher expression levels of the biomarkers after stimulation with sensitizing chemicals when compared to THP-1 and MUTZ-3 cells.

U937 cells lack ARNT expression and, as expected, showed no increased expression of CYP1A1, CYP1B1 or IL-1β after stimulation with benzo[a]anthracene.

A statistically significant expression of CYP1A1 and CYP1B1 by benzo[a]anthracene could be observed for MUTZ-LC. However, the increase of expression was much lower compared to iDC, LC or monocytes.

On the other hand, MUTZ-DC showed a strong CYP1A1 expression after stimulation with benzo[a]anthracene. Nickel sulphate exhibited a stimulatory capacity in MUTZ-3-DC with regard to cytokine mRNA induction, but not surface marker expression compared to $CD34^+$ DC (Nelissen et al., 2009). Recently, MUTZ-3-DCs and moDCs were used for transcriptional profiling to describe and assess their functionality and applicability with regard to DC-based *in vitro* assays for prediction of sensitization and DC-based immunotherapy (Lundberg et al., 2013). Both cell types were most closely related to primary $CD1c^+$ myeloid dendritic cells (mDCs) isolated from tonsillar tissue, but a large number of immune-related transcripts were found to differ across moDCs and MUTZ-3-DCs, suggesting different applicability in test assays. Furthermore, iDC display a different expression profile of the AhR-independent metabolizing enzymes CYP2S1, CYP2E1 and CYP3A5 than the myeloid cell lines, indicating that these cell types are able to metabolize various xenobiotic compounds.

Besides CYP1A1, CYP1B1 and IL-1β, additional AhR target genes are known, including UGT1A8, UGT1A10 (Cheng et al., 2012), MRP4 (Xu et al., 2010) and possibly CYP2A6 (Abu-Bakar et al., 2012). Smith et al. (2003) showed that topical coal tar induces cutaneous CYP2S1 expression *in vivo* and identified multiple xenobiotic-response-elements consensus sequences in the CYP2S1 promotor, leading them to the prediction that PAHs are also metabolized by CYP2S1. Yeager et al. (2009) demonstrated that Nrf2, in addition to AhR, is required for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induction of classical AhR battery genes Nqo1, Ugt1a6, and Gsta1, as well as most Ugt and Gst isoforms in liver of mice. AhR and Nrf2 are transcription factors that control Nqo1/NQO1 expression (Lin et al., 2011). It is hypothesized that NQO1 induction occurs via AhR-dependent Nrf2 activation (Haarmann-Stemmann et al., 2012). Ott et al. (2010) and Python et al. (2009) have identified NQO1 as a marker gene up-regulated after stimulation of moDCs with cinnamic aldehyde by microarray analysis. NQO1 was modulated by most sensitizers after exposure of MUTZ-3 cells and PBMDCs (peripheral blood mononuclear-derived dendritic cells) with selected sensitizers for 24 h (Python et al., 2009). NQO1 expression was also affected by β-lactam antibiotics in moDCs, suggesting a direct role of AhR signal transduction in contact hypersensitivity reactions (Sebastian et al., 2012). Jux et al. (2009) found that $Ahr^{-/-}$ mice mounted a lower contact hypersensitivity response against FITC but it appeared not due to an inability of LC or dermal DC to leave the skin and reach the draining lymph nodes after antigen uptake.

The different levels of AhR and ARNT expression measured in this study and the different levels of induced AhR-dependent gene expression indicate that some cell lines may have a more limited applicability domain for assessment of skin sensitizing chemicals than others. It is therefore critically important to investigate the metabolic capability of cell lines used in *in vitro* models for the prediction of skin sensitization. Otherwise, allergens, especially pro-haptens, could escape APC-based *in vitro* detection. Additional studies are required regarding the combination of cutaneous xenobiotic metabolizing enzymes and APC-sensitization for the development of valid *in vitro* models for skin sensitization assessment.

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Conflict of interest

The authors state no conflict of interest.

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