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Benzo[*a*]pyrene affects Jurkat T cells in the activated state via the antioxidant response element dependent Nrf2 pathway leading to decreased IL-2 secretion and redirecting glutamine metabolism

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ABSTRACT

There is a clear evidence that environmental pollutants, such as benzo[*a*]pyrene (B[*a*]P), can have detrimental effects on the immune system, whereas the underlying mechanisms still remain elusive. Jurkat T cells share many properties with native T lymphocytes and therefore are an appropriate model to analyze the effects of environmental pollutants on T cells and their activation. Since environmental compounds frequently occur at low, not acute toxic concentrations, we analyzed the effects of two subtoxic concentrations, 50 nM and 5 μM, on non- and activated cells. B[*a*]P interferes directly with the stimulation process as proven by an altered IL-2 secretion. Furthermore, B[*a*]P exposure results in significant proteomic changes as shown by DIGE analysis. Pathway analysis revealed an involvement of the AhR independent Nrf2 pathway in the altered processes observed in unstimulated and stimulated cells. A participation of the Nrf2 pathway in the change of IL-2 secretion was confirmed by exposing cells to the Nrf2 activator tBHQ. tBHQ and 5 μM B[*a*]P caused similar alterations of IL-2 secretion and glutamine/glutamate metabolism. Moreover, the proteome changes in unstimulated cells point towards a modified regulation of the cytoskeleton and cellular stress response, which was proven by western blotting. Additionally, there is a strong evidence for alterations in metabolic pathways caused by B[*a*]P exposure in stimulated cells. Especially the glutamine/glutamate metabolism was indicated by proteome pathway analysis and validated by metabolite measurements. The detrimental effects were slightly enhanced in stimulated cells, suggesting that stimulated cells are more vulnerable to the environmental pollutant model compound B[*a*]P.

Introduction

There is an epidemiological (Sopori, 2002) and experimental (Galvan et al., 2006) evidence that environmental pollutants, such as polycyclic aromatic hydrocarbons (PAHs), can have a detrimental effect on the immune system. Benzo[*a*]pyrene (B[*a*]P), a representative PAH, and its metabolites influence both cell mediated and humoral mediated immunity (Urso et al., 1986), but the mechanism how B[*a*]P affects the cells of the immune system is largely unknown. B[*a*]P is reported to have a direct toxic impact through the induction of DNA damage in cells (Briede et al., 2004). Typically, this process is initiated by the binding of B[*a*]P to the aryl hydrocarbon receptor (AhR). This complex is then translocated to the nucleus, where it interacts with the ARNT protein (Puga et al., 2000). The built heterodimer acts as a transcription factor that binds to xenobiotic response elements (XRE), which regulate the transcription of genes coding for proteins involved in the primary (Shimada et al., 1989) and secondary metabolism (Hankinson, 1995; Kohle and Bock, 2007) of xenobiotics. The induced metabolism leads to the conversion of B[*a*]P to several metabolites. 7alpha,8beta-di-hydroxy-9beta,10beta-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE) is among the formed substances and is reported to form covalent DNA-adducts and causes genotoxic effects (Jack and Brookes, 1980; Watson et al., 1985).

It was shown that other PAHs, such as 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), can alter the differentiation process of epithelial (Lew et al., 2009) and immune cells (De Abrew et al., 2011). Since the immune response dependents on a sensitive balance between many different cells, which activate, enhance and suppress each other's activities, even slight changes can have a major impact on the immune system homeostasis.

The expression of AhR varies among the different subtypes of immune cells. The receptor is often not detected due to its absence or

Abbreviations: AhR, aryl hydrocarbon receptor; ARE, antioxidant response element; B[*a*]P, benzo[*a*]pyrene; IL-2, Interleukin-2; tBHQ, *tert*-butylhydroquinone; TCDD-2,3,7,8, Tetrachlorodibenzo-*p*-dioxin.

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sensitivity limits of the assay (Stockinger et al., 2011). More specifically, Ah receptor expression is not detectable in different T cell subsets, such as T helper cell 1 (Th1) and Th2, but high levels of AhR are expressed in Th17 cells (Esser et al., 2009; Veldhoen and Duarte, 2010). Therefore it is relevant how environmental contaminants, such as B[*a*]P, influence immune cells in an AhR independent manner. Since Jurkat T cells do not express the AhR, they are an ideal model system to study the effects of environmental contaminants on immune cells independent of AhR signaling.

The second most important pathway, which may be involved in mediating the toxic effects of PAHs, is the Nrf2 oxidative stress response signaling. PAHs, such as B[*a*]P, have been shown to alter the transcriptional activity of Nrf2. Electrophilic compounds can cause oxidative stress either directly or indirectly through the oxidation of KEAP. Due to oxidation KEAP loses its ability to sequester Nrf2 in the cytosol. The dissociated Nrf2 then translocates into the nucleus, where it binds and activates the antioxidant/electrophile response element (ARE/EpRE), which is responsible for the transcription of second phase detoxification enzymes (Burczynski and Penning, 2000; Miller et al., 2000), and other proteins (Garg et al., 2008; Tkachev et al., 2011). In particular, ARE regulates proteins involved in hydrolysis (carbonyl esterase), reduction (carbonyl reductase), oxidation (aldehyde dehydrogenase), glucuronidation pathway (UDP-glucose dehydrogenase) and glutathione synthesis (GCS, regulatory subunit) as well as glutathione transferases (GST class mu), antioxidants (glutathione peroxidase), protective proteins (multidrug resistance protein), NADPH regenerating enzymes (G6PDH), inflammatory suppressive genes (glucocorticoid-regulated kinase) and others that cannot be summed up in a common group like tryptophan hydrolase (Thimmulappa et al., 2002). Recently, several more ARE controlled genes were identified in a microarray based study (Wen et al., 2011).

Cell line based experimental systems can provide insights into distinct cellular functions. This helps to generate hypotheses that can be tested in a limited number of experiments in primary cells or even animals afterwards. One of the most widely used cellular T cell models are Jurkat T cells. They were also chosen for this study because they share many features with native immune cells and do not express the Ah receptor (Abraham and Weiss, 2004; Nguyen et al., 2010). The induction of IL-2 secretion upon stimulation with PMA/ionomycin is another similarity between native and Jurkat T cells (Higai et al., 2009; Tanaka et al., 2005). The stimulation causes an increase in secreted IL-2 of about three orders of magnitude, which can be even more pronounced in native T cells. The controllable stimulation of Jurkat T cells offers the possibility to analyze the toxic effects of B[*a*]P not only on fully activated cells but also on the dynamic process of activation. As the activation of immune cells by other cells or antigens is a crucial process in the immune response, the effects of B[*a*]P on this process are of special interest.

Environmental compounds occur very often at low, not acute toxic concentrations and therefore we decided to analyze the effects of two subacute concentrations (50 nM and 5 μM). Additional to the absence of the Ah receptor, Jurkat T cells have been shown to possess no B[*a*]P metabolites or enzymatic activities related to B[*a*]P metabolism (Nguyen et al., 2010). Since the formation of toxic metabolites is impaired, it is assumed that the absence of the AhR significantly lowers the sensitivity towards toxins. Hepatocytes express the Ah receptor and a concentration of 5 μM was shown to directly cause toxic effects (Dautel et al., 2011).

In order to elucidate the molecular mechanisms by which environmental compounds mediate their effects, a system wide analysis of the key molecules in the cells is beneficial. In the recently established field of toxicoproteomics (Huang et al., 2011; Kodavanti et al., 2011; Kroger et al., 2004) changes in the proteome are detected, and the significantly regulated proteins are used for a pathway enrichment process. The simultaneous alteration of several proteins in a given pathway strongly supports the assumption that this particular pathway is involved in the molecular response towards the exposure with an environmental compound.

The objective of this study was to identify the mode of action of B[*a*]P in an AhR-independent but stimulation dependent manner in Jurkat T cells. The applied unspecific activation allows obtaining first insights which can be used in follow-up studies for the simplified analysis of processes involved in specific activation.

Material and methods

Jurkat T cell culture and stimulation. Jurkat T cells (clone E6-1, TIB-152, LGC Promochem, Wesel, Germany) were routinely maintained in RPMI-1640 medium containing 10% fetal bovine serum, 1% L-glutamine (all from Biochrom AG, Berlin, Germany), 1% penicillin (100 U/ml)/streptomycin (100 mg/ml) (PAA, Pasching, Austria) at an atmosphere of 5% CO_2 and 95% humidity at 37 °C in a CO_2 incubator (MCO-18AIC, Sanyo Electric Co Ltd., Gunma-ken, Japan). Jurkat T cells were seeded at a density of 1×10^6 cells/ml in 25 cm^2 flasks (Greiner Bio-One, Solingen, Germany). 24 h later, cell stimulation was induced by the addition of 10 ng/ml phorbol-12-myristat-13-acetate (PMA) and 1 μg/ml ionomycin (IO, both from Sigma-Aldrich, Steinheim, Germany) for 4 h. T cell stimulation was assessed by monitoring the expression of the surface marker CD69. Briefly, about 1×10^6 cells were incubated with 2.5 μl CD69PE antibody (Immunotech/Coulter, Krefeld, Germany) for 30 min. Excessive unreacted antibody was removed by washing with PBS (Biochrom AG., Berlin, Germany). Cells were resuspended in 200 μl of fixation buffer (2% Paraformaldehyde in PBS) and analyzed with flow cytometry (BD FACSCalibur system) using BD CellQuest Pro software.

Exposure of B[a]P and protein extraction. Jurkat T cells were exposed to B[*a*]P (50 nM and 5 μM) for 4, 24 and 48 h. Benzo[*a*]pyrene of ≥96% (HPLC) purity (Sigma-Aldrich, Steinheim, Germany) was dissolved in DMSO (Applichem, Darmstadt, Germany) to obtain a 10 mM stock solution. Control cells were exposed to DMSO without the addition of B[*a*]P and all experiments were performed in triplicates. After the indicated times, the cells were harvested and washed twice with PBS. The cytosolic proteins were extracted and protein estimation was carried out as described previously (Morbt et al., 2009).

In order to validate the involvement of the Nrf2 pathway in the different signaling after exposure to B[*a*]P Jurkat T cells were treated with *tert*-butylhydroquinone (tBHQ), a known activator of the Nrf2 pathway (Rockwell et al., 2012). tBHQ (Sigma-Aldrich, Steinheim, Germany) was dissolved in DMSO resulting in a 100 mM stock solution. After activation with PMA and Ionomycin for 4 h, the cells were incubated for 24 h and 48 h with either tBHQ (50 μM final concentration), B[*a*]P (5 μM final concentration) or both, tBHQ and B[*a*]P. Control cells were exposed to DMSO and all treatments were performed in triplicates. After the cell harvest, the supernatants were removed and stored at −20 °C.

ELISA measurement of IL-2 in culture supernatants. Detection of IL-2 in culture supernatants was performed in triplicates by sandwich ELISA using 384-well plates (MaxiSorp, Nunc, Langenselbold, Germany) and the BD OptEIA Human IL-2 ELISA kit according to the manufacturer's protocol (BD Biosciences, Heidelberg, Germany). Briefly, ELISA plates were precoated with 50 μl of 1:500 diluted capture antibody overnight at 4 °C and afterwards washed and blocked with 100 μl assay diluent (PBS with 10% FCS) at room temperature for 1 h. After the washing step, 50 μl standard and culture supernatant (1:4 diluted with PBS) were incubated at room temperature for 1 h, washed and 50 μl detection antibody and streptavidin-HRP reagent were added and incubated at room temperature for 1 h. After subsequent washing steps 50 μl substrate solution was added and incubated in the dark for 10–30 min. The reaction was stopped by the addition of 50 μl 2N H_2SO_4. The measurement was performed using an ELISA reader (Infinite F200, Tecan, Männedorf, Switzerland) and the data evaluation was carried out using the Magellan software (Magellan, Tecan, Crailsheim, Germany).