In vitro research work

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1. Background

The present review highlights the most relevant in vitro-research work dealing with fine particulate and air pollutants done in Austria during the last decade. As the public and scientific awareness of health impairment due to ambient pollutants is rising, several national scientific groups specialized on diverse topics concerning air pollutants with main focus on the effects of cigarette smoke components. Obviously, both scientific know-how and technical equipment in this research field are available in this country to be competitive at an international level, as several publications show that are described below. In order to ensure a high level of research quality, only papers published in a peer-reviewed journal and listed in the PubMed database were taken into consideration.

In general, the definition of in vitro and in vivo-research refers to the applied experimental model. Therefore, in vitro-research is the manipulation of tissues, cells or biomolecules in a controlled, artificial environment, serving as an approximate reconstruction of a living plant, animal or human being (in vivo). The great benefits of this method are the possibility to modify single variables of any biological process and analyze the effects of modifications in a highly standardized, rather inexpensive, and feasible way.

2. Cigarette smoke components

Cadmium

Environmental pollutants are a very delicate public health issue with adverse health effects, ranging from asthma to increased mortality rates. As a fact, airborne particles containing organic and inorganic substances in low doses activate the immunological chain reaction by influencing the chemokine production of immunocompetent cells and further relevant cell types such as epithelium and endothelium (Chauhan et al. 2005). For example, the trace element cadmium (Cd) found in cigarette smoke shows acute and chronic health effects including immune system alterations. Messner and colleagues showed with in vitro-experiments that Cd is a promoter of atherosclerotic lesions by increasing endothelial cell permeability and inhibition of cell proliferation (Messner et al. 2009). Considering this immunological background, Marth et al. performed an elegant in vitro-study to assess the interaction of pollutants with the gene expression of various cytokines that are important for cell

communication and immunomodulation (Marth et al. 2000). Peripheral blood mononuclear cells were exposed to cadmium chloride ($CdCl_2$). Afterwards, the keyplayer-cytokines beta-actin, ICAM-1, IL-1alpha, IL-6, IL-4, TNF-alpha, IL-4 and IFN-gamma as well as the heat shock protein HSP70 were quantified. Especially HSP70 is a suitable marker for cell reaction to pathophysiological and environmental stress (Ciocca et al. 1993). As a result, $CdCl_2$ was able to stimulate these components of the human immune system at low concentrations, whereas a higher dose had an immunosuppressing effect. Based on this work, the authors showed that low doses of cadmium were also able to inhibit the production of the immunoglobulin IgE by stimulated human B cells, but enhanced the amount of secreted IgE and did not alter IgM release (Jelovcan et al. 2003). In conclusion, these observations were able to give an explanation for the inflammatory and mucous swelling effects of pollutants such as heavy metals in the airways.

Standardized cigarette smoke extract

Cigarette smoking is known to have various detrimental health effects, including allergic airways symptoms. To investigate the pathophysiological mechanisms of the development of allergic disease, Gangl et al. incubated cultured human bronchial epithelial cells with ingredients of cigarette smoke in order to mimic the in vivo-situation of exposure with active and passive smoking (Gangl et al. 2009). Interestingly, in this setting, the treatment with smoke extract weakened the epithelial cells, leading to a more than threefold increase of cell permeability for some of the major respiratory allergens (Bet v1, Phl p5, Der p2) and a subsequent stimulation of histamine production in histamine release experiments. These results indicate that the substances found in cigarette smoke reduce the barrier function of the respiratory epithelium for allergens and, thus, may cause or exacerbate allergic inflammation.

Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are abundantly found in cigarette smoke and diesel exhaust. In a recent publication, the possible immunotoxic effects of PAHs were evaluated with a screening assay using transfected human cell lines and subsequent measurement of luciferase activity (Oostingh et al. 2008). For this screening method, Oosthing et al. used a panel of chemokines and cytokines that were administered to cultured T cells (Jurkat) and lung epithelial cells (A549). Both cell types are crucial in immune defense. The PAH fluoranthene was applied to establish the assay. This substance has been found to contribute to the increasing incidence of allergic diseases in humans and rodents (Kanoh et al. 1996, Polosa et al. 2002). Furthermore, the in vitro-effects of the wide-spread PAHs phenanthrene, pyrene, and anthracene were

evaluated. As a pro-inflammatory standardized factor, recombinant human TNF-alpha was used to simulate a rather initial immune process resulting in a vast chemokine production. The authors stressed that their newly developed assay would be a useful system for the screening of various substances in a cheap, simple, and easy manner. What is more, it could serve as an alternative for animal testing of any ambient toxins.

3. Allergens

A Viennese research group concentrated on the role of allergen-specific T cell-priming in cord blood from women that were exposed to both inhalant and nutritive allergens during gestation. In a first attempt, a placenta model was used consisting of in vitro-perfused placentas from uncomplicated term pregnancies to directly examine transplacental transfer of two chosen substances, the milk allergen beta-lactoglobin and the birch pollen major allergen Bet v1 (Szepfalusi et al. 2000, Schneider et al. 1972, Reisenberger et al. 1996, Fortunato et al. 1992). These allergens were able to cross the maternofetal barriers and could be detected in the fetal circulation. Furthermore, cord blood mononuclear cells were incubated with birch pollen allergen and timothy grass allergen (Szepfalusi et al. 2000). Simultaneously, maternal blood samples were treated equally to rule out the influence of maternal exposition to inhalable allergens. The data suggest that these allergens have a positive effect of fetal lymphoproliferative activity at gestational age of 20 weeks, supporting the idea of a modulatory function of the placental barrier concerning the T cell-response to allergen contact.

4. Halogenated substances

The aryl hydrocarbonon receptor (AhR) is a ligand-activated nuclear transcription factor that mediates responses to halogenated aromatic toxins such as the so-called xenodioxin 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), polynuclear aromatic hydrocarbons, combustion products, and numerous chemoprotective phytochemicals such as flavonoids. TCDD induces tumors in animals and in humans, whereas natural AhR ligands tend to protect cancer. Both TCDD and phytochemicals inhibit estrogen-induced breast and endometrial neoplasms, suggesting that the AhR can provide an important target for development of therapeutic drugs for treating these cancers (Safe 2001). Schaufler et al. investigated the effects of TCDD in vitro on hormone-resistant prostate cancer cell lines (Schaufler et al. 2002). As the expression of the phase 1 and 2 metabolism genes of the cytochrome P450 superfamily CYP1A1 and CYP1A2 are regulated by AhR, they were used as unspecific markers for AhR activation (Hu et al. 2007). It was shown that cell growth was not altered, whereas CYP1A1 and CYP1B1 were significantly induced in high passage num-

bers, pointing towards a potential role of an environmental toxin in causing a less effective drug therapy in advanced states of prostate cancer due to CYP induction.

5. Conclusion and outlook

Research on environmental and air pollutants is of high importance considering that air quality is a key environmental concern for quality of life and public health. Besides monitoring networks and data collection of air pollution, in vitro-research is essentially needed to fully understand how the various ambient air pollutants behave and affect the environment and life forms on a molecular level. Interdisciplinary cooperation and communication should be strengthened in the future to ensure a broader scientific as well as public discussion of important findings of in vitro research work on pollutants in Austria.

6. References

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