## Abstract

Allergic contact dermatitis (ACD) is considered to be the most prevalent form of immunotoxicity in humans, therefore it is an important endpoint taken into account in the assessment of the hazard and risk of chemicals. ACD may be induced by chemicals, fragrances and preservatives, the increase in the incidence of allergic diseases is also correlated with the increase in industrialization and environmental pollution. Common environmental sensitizing substances and pollutants include: diesel exhaust particulate (DEP); plastic, rubber and glue components; preservatives; metals; medicines; microplastic (MP), and nanoplastic (NP).

Traditionally, the identification and characterization of skin sensitizing chemicals has required the use of *in vivo* testing. Currently, in order to limit animal experiments, alternative methods are used based on the so-called Adverse outcome pathways (AOPs), which are currently a key component of human risk assessment in predictive toxicology. Currently used alternative methods approved by the Organization for Economic Co-operation and Development (OECD) address four key elements in the mechanism of skin sensitization: i) molecular interaction with skin proteins; ii) keratinocyte inflammatory response (KC); iii) activation of dendritic cells (DC); iv) lymphocyte proliferation. Each of the key elements has a separate test method. Therefore, it is necessary to perform a combination of several tests to obtain information equivalent to *in vivo* tests allowing the classification of the test substance as sensitizing or non-sensitizing.

The aim of the dissertation was to develop an *in vitro* method assessing the sensitizing potential of the tested substances, which consolidates at least two key events of the allergic reaction, i.e. the keratinocyte inflammatory reaction (KC) and the activation of dendritic cells (DC).

The aim of the research was to determine the endpoints of the method appropriate for keratinocytes and dendritic cells and to define the method of co-culture for these two types of cells. In the first stage of the work, the profile of the release of selected inflammatory cytokines by 3 types of keratinocytes after exposure to a sensitizing substance was verified. The production of cytokines was found to be dependent on cell type, cell concentration and the duration of cell exposure. In the next stage of work, it was verified whether the level of inflammatory cytokines released by keratinocytes was influenced by the presence of dendritic cells. The result of the first stages of the research was the selection of two types of cells, i.e. neonatal keratinocytes (NHEK-neonatal) and DC-like THP-1 cells as the most appropriate

system of cells cultured in a co-culture. Two inflammatory cytokines, i.e. IL-1 alpha and IL-18, were also selected as potential endpoints of the final method. In the third stage of the study, the influence of the method of co-culturing (indirect co-culture, direct co-culture) keratinocytes and dendritic cells on the expression of surface antigens on dendritic cells was assessed. The results of this step showed that the optimal type of keratinocyte and dendritic cell co-culture is direct co-culture and the CD54 surface antigen can be used as the end point in the final co-culture method.

Based on the experiments carried out, the following conditions of the final KC/DC co-culture method were determined:

- keratinocytes: NHEK-neonatal,
- dendritic cells: THP-1,
- type of co-culture: direct,
- exposure time to the tested substances: 24 h,
- endpoints: measurement of IL-1 alpha and IL-18 concentration in post-culture media by ELISA-xMAP method and evaluation of CD54 antigen expression on THP-1 cells by flow cytometry.

In the last stage of the works, the ability of the developed method to assess the sensitizing potential of 14 substances known to be sensitizing and non-sensitizing was verified. It has been shown that the best predictive model for assessing sensitization potential is the model using the assessment of CD54 antigen expression and the level of IL-18 in post-culture media. Using this predictive model, all sensitizing substances tested (9/9) and four of the five non-sensitizing substances (4/5) were correctly classified.

The results of the research confirmed the main thesis of the dissertation on the possibility of using a method that integrates two key events, i.e. the inflammatory reaction of keratinocytes and activation of dendritic cells for the assessment of skin sensitization potential. Additionally, a greater potential of the co-culture method for the detection of substances requiring metabolic activation (prohaptens) was found compared to the monoculture method.