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THP-1 monocytes but not macrophages as a potential alternative for CD34$^+$ dendritic cells to identify chemical skin sensitizers

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ABSTRACT

Early detection of the sensitizing potential of chemicals is an emerging issue for chemical, pharmaceutical and cosmetic industries. In our institute, an *in vitro* classification model for prediction of chemical-induced skin sensitization based on gene expression signatures in human CD34$^+$ progenitor-derived dendritic cells (DC) has been developed. This primary cell model is able to closely mimic the induction phase of sensitization by Langerhans cells in the skin, but it has drawbacks, such as the availability of cord blood.
The aim of this study was to investigate whether human *in vitro* cultured THP-1 monocytes or macrophages display a similar expression profile for 13 predictive gene markers previously identified in DC and whether they also possess a discriminating capacity towards skin sensitizers and non-sensitizers based on these marker genes. To this end, the cell models were exposed to 5 skin sensitizers (ammonium hexachloroplatinate IV, 1-chloro-2,4-dinitrobenzene, eugenol, *para*-phenylenediamine, and tetramethylthiuram disulfide) and 5 non-sensitizers (L-glutamic acid, methyl salicylate, sodium dodecyl sulfate, tributyltin chloride, and zinc sulfate) for 6, 10, and 24 h, and mRNA expression of the 13 genes was analyzed using real-time RT-PCR.
The transcriptional response of 7 out of 13 genes in THP-1 monocytes was significantly correlated with DC, whereas only 2 out of 13 genes in THP-1 macrophages. After a cross-validation of a discriminant analysis of the gene expression profiles in the THP-1 monocytes, this cell model demonstrated to also have a capacity to distinguish skin sensitizers from non-sensitizers. However, the DC model was superior to the monocyte model for discrimination of (non-)sensitizing chemicals.

Introduction

Many low-molecular weight (LMW) compounds give rise to allergic contact dermatitis. Up till now, the most commonly used and officially validated test to identify skin sensitizing potency of chemicals is the murine local lymph node assay (LLNA) (OECD, 2002). Many researchers have explored several approaches in developing an *in vitro* skin sensitization test, which were mainly based on the use of relevant cell models, such as keratinocytes, Langerhans cells (LC), dendritic cells (DC), co-culture systems of these cells, and even human skin explants or equivalents (Ryan et al., 2001).

Skin LC play a critical role in the induction phase of allergic contact hypersensitivity. Upon antigen capture, LC differentiate, mature, and migrate to the draining lymph nodes where they present the allergens to naive T cells and trigger their proliferation (Hart, 1997; Banchereau and Steinman, 1998). LC maturation is characterized by expression of major histocompatibility complex class II and co-stimulatory molecules, such as CD40, CD54, CD83, and CD86 (Ozawa et al., 1996; Verrier et al., 1999), as well as production of cytokines, for example interleukin (IL)-1β and IL-6 (Enk and Katz, 1992; Cumberbatch et al., 1996). To date, however, the use of *ex vivo* LC has been restricted due to the difficulty of obtaining a sufficient number of LC from the epidermis, their weak viability, the shortage of available human skin, and the spontaneous maturation of LC once cultured. Possible sources of LC-like cells are peripheral blood (monocyte-derived dendritic cells (Mo-DC)) (Sallusto and Lanzavecchia, 1994) and bone marrow or cord blood (CD34$^+$ progenitor-derived dendritic cells (CD34-DC)) (Caux et al., 1992). Chemical allergens (e.g. 1-chloro-2,4-dinitrobenzene

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(DNCB) and nickel sulfate ($NiSO_4$)) have been demonstrated to directly induce *in vitro* phenotypic alterations (e.g. CD54, CD86, and HLA-DR) and cytokine production (e.g. tumor necrosis factor (TNF)-α, IL-1β, and IL-8) in Mo-DC and CD34-DC (Aiba et al., 1997, 2003; Coutant et al., 1999; De Smedt et al., 2001, 2002; Staquet et al., 2004). Besides analyses of surface marker expression and cytokine production, changes in gene expression have been studied to reveal new biomarkers that are suitable for developing an *in vitro* alternative for skin sensitization (Ryan et al., 2004; Schoeters et al., 2005, 2006, 2007; Verheyen et al., 2005). Based on the differential expression of a subset of 13 gene markers after exposure of CD34-DC to 21 chemicals, a classification model for predicting the skin sensitizing capacity was developed (Hooyberghs et al., 2008). Both phenotypic and genotypic studies on primary dendritic cells (DC) suggested that the key steps involved in DC maturation can be reproduced *in vitro*. The use of primary DC for identifying chemical allergens has potential, but there are still some technical limitations concerning the routine use of these cells in skin sensitization tests, such as the availability of human peripheral or cord blood, the ethical requirements with respect to the informed consent, and donor to donor variability (Aiba et al., 1997; Coutant et al., 1999; Rougier et al., 2000; Tuschl et al., 2000). A possible alternative may be based on human myeloid leukaemia cell lines. The most intensively studied myeloid cell lines in the context of skin sensitization are KG-1, MUTZ-3, THP-1, and U-937 (Hulette et al., 2001; Ashikaga et al., 2002; Hulette et al., 2002; Azam et al., 2006; Python et al., 2007). These cell lines can be routinely cultured and display various key *in vivo* DC characteristics, such as esterase activity, lysozyme production, and phagocytosis (Tsuchiya et al., 1980) (Sundstrom and Nilsson, 1976). Ashikaga et al. (2006) developed an *in vitro* predictive method for assessing the sensitizing potential of chemicals by measuring the differential expression of CD54 and CD86 on the surface of THP-1 cells (human Cell Line Activation Test; h-CLAT) (Ashikaga et al., 2006; Sakaguchi et al., 2006). Although not yet validated, the h-CLAT is a promising model and is currently under evaluation for validation. Miyazawa et al. (2007) confirmed that THP-1 cells are suitable as LC surrogates, by measuring markers reflecting DC maturation, e.g. CD1a, CD40, CD54, CD58, CD83, CD86, IL-6, IL-8, and TNF-α (Miyazawa et al., 2007). Other researchers established resemblance between allergen-induced Mo-DC and THP-1 monocytes regarding maturation markers (Bocchietto et al., 2007; Tietze and Blomeke, 2008). The THP-1 monocytes can also be induced to differentiate into macrophage-like cells by phorbol 12-myristate 13-acetate (PMA) (Auwerx, 1991). DC are the most prominent antigen-presenting cells (APC), but macrophages also exhibit the capacity to process antigens (Holt, 2000). Macrophages secrete cytokines and chemokines, including chemokine (CC motif) ligand (CCL)2, CCL3, chemokine (CXC motif) ligand (CXCL)8, interferon-γ, IL-1, IL-6, and TNF-α (Gosset et al., 1999; Rosenwasser, 2000), which results in the recruitment and activation of other inflammatory cells (Vissers et al., 2005). However, they may also inhibit allergic inflammation through the secretion of various inhibitory mediators, including IL-10 and prostaglandin E2 (PGE2) (Bloemen et al., 2007). Macrophages originating from myeloid lineage, may exhibit some important phenotypic features similar to DC. They increase expression of the same cell surface antigens after lipopolysaccharide stimulation (Cao et al., 2005; Liu et al., 2006) and, under certain conditions, differentiate into DC-like cells (Lee et al., 2004). In addition, a correlation between allergen-induced IL-1β synthesis in macrophages and the immunological response in the LLNA of a given compound has been established (Arkusz et al., 2007).

In this study, we tested whether human *in vitro* cultured THP-1 monocytes and macrophages resemble the expression profile of 13 gene markers for skin sensitization in CD34-DC (Hooyberghs et al., 2008). Subsequently, the discriminating capacity between chemical skin sensitizers and non-sensitizers based on the gene transcript profile of the most resembling cell line model was compared to the primary CD34-DC model. The results of CD34-DC were obtained in a previous research performed by Hooyberghs et al. (2008).

Methods

Chemical compounds. The selection of chemical compounds for this preliminary study was based on a number of criteria. For reasons of comparison, the chemicals needed to be a subset of the 21 compounds to which CD34-DC were previously exposed and for which *in vivo* sensitization data were available (Hooyberghs et al., 2008). A random selection of 10 out of these 21 chemicals was investigated here. Further, an equilibrium in the number of skin sensitizers versus non-sensitizers was maintained by testing 5 skin sensitizing chemicals (ammonium hexachloroplatinate IV (HCPt), DNCB, 2-methoxy-4-(2-propenyl)-phenol or eugenol (Eug), *para*-phenylenediamine (*p*PD), tetramethylthiuram disulfide (TMTD)) and 5 non-sensitizing compounds (L-glutamic acid (L-GA), methyl salicylate (MeSA), sodium dodecyl sulfate (SDS), tributyltin chloride (TBT), and zinc sulfate ($ZnSO_4$)). All chemicals were purchased from Sigma-Aldrich Chemie Gmbh (Steinheim, Germany), except for dimethylsulfoxide (DMSO, LabScan Ltd., Dublin, Ireland).

For THP-1 macrophages, data were obtained for only 8 out of 10 chemicals (HCPt, DNCB, *p*PD, TMTD, L-GA, MeSA, SDS, and TBT).

Cell culture. THP-1, a human monocytic leukaemia cell line, was obtained from American Type Culture Collection (LGC Promochem, Teddington, UK). THP-1 cells were cultured in complete culture medium containing 89% RPMI 1640 Medium with GlutaMAX™-1 and 25 mM HEPES (Invitrogen, Paisley, UK) supplemented with 10% fetal bovine serum (Biochrom AG, Berlin, Germany) and 1% Penicillin (5000 U/ml)–Streptomycin (5000 μg/ml) (Invitrogen). The cells were kept in a humidified atmosphere at 37 °C and 5% CO_2. They were subcultured every 3–4 days. When the cells were at a concentration of 0.8×10^6 cells/ml/well, they were exposed to the test substance.

To obtain the THP-1-derived macrophages, THP-1 monocytes were seeded in 6-well plates at 1.25×10^6 cells/ml/well complete medium and were incubated with 0.25 mg/ml PMA (Sigma-Aldrich Chemie Gmbh) for 48 h to allow adherence and differentiation before exposure to the test substance (Auwerx, 1991).

Results of CD34-DC were obtained in a previous research (Hooyberghs et al., 2008) and the culture method was described in detail by Schoeters et al. (2007).

Chemical exposure of the cells. THP-1 monocytes and macrophages were exposed in 6-well plates to the chemicals (all from Sigma-Aldrich Chemie Gmbh) for 6, 10 and 24 h. The concentrations used in exposure experiments yielded 25% cell death (IC25) for THP-1 monocytes and 20% cell growth inhibition (IC20) for THP-1 macrophages at 24 h. In THP-1 monocytes this was previously determined on at least 3 biological replicates using propidium iodide staining which was evaluated by flow cytometry (FACSCalibur, BD Biosciences, Erembodegem, Belgium). Cell growth inhibition of exposed THP-1 macrophages was assessed by neutral red experiments. Since no cytotoxicity was found for MeSA and L-GA, these compounds were used at the highest soluble concentration. The exposure concentrations are summarized in Table 1. For each chemical and its corresponding solvent, 3 independent exposure experiments were performed. Exposure of CD34-DC was performed for each chemical and its corresponding solvent, derived from at least 3 independent donors (except for DNCB only 2) in previous research (Hooyberghs et al., 2008).

The chemicals were dissolved in DMSO (LabScan Ltd.) or the respective culture medium (Table 1). The final concentration of DMSO was 0.1% and 0.05% in the medium of monocytes and macrophages, respectively. In the comparison analysis of the different cell types, 4 skin sensitizers (DNCB, HCPt, *p*PD, TMTD) and 4 non-sensitizers (L-