

Eschrich D.<sup>1</sup>, Engels U.<sup>1</sup>, Scheel J.<sup>2</sup> and K. Schroeder<sup>1</sup>

<sup>1</sup> Phenion GmbH & Co. KG, Düsseldorf, Germany; <sup>2</sup> Henkel KGaA, Corporate SHE and Product Safety/Human Safety Assessment, Düsseldorf, Germany  
dietmar.eschrich@henkel.com

## Introduction

- Allergic contact dermatitis (ACD) resulting from skin sensitization is an allergic skin reaction after contact with a substance.
- Dendritic cells of the skin (Langerhans cells) play a key role in the development of ACD. After the uptake of a chemical allergen they become activated, migrate to the local lymph node and induce a specific T lymphocyte response.
- Upregulation of CD86 expression is a hallmark of dendritic cell activation and maturation.
- Langerhans cells are difficult to isolate in an adequate quantity and quality. Therefore, monocyte derived dendritic cells (MoDC) are discussed as Langerhans cell surrogates to predict skin sensitization in vitro.
- We have examined the use of MoDC with the well know maturation marker CD86 as a tool to predict skin sensitization in vitro.

## Methods

**Generation of MoDCs:** Peripheral blood mononuclear cells (PBMCs) were separated by Ficoll-Hypaque (PAA Laboratories GmbH) gradient centrifugation of buffy coats (Univerty of Düsseldorf, Blutspendezentrale). Monocytes were isolated from PBMCs by negative selection using the Monocyte isolation kit II (Miltenyi Biotec, Bergisch Gladbach) according to the manufacturer's instructions. For generating MoDCs monocytes were incubated for 5 days in medium supplemented with rhIL4 (500 U/ml) and rhGM-CSF (200 U/ml). To evaluate the purity and quantity of monocytes and MoDCs staining with FITC conjugated anti-CD1a and anti-CD14 (BD Pharmingen) was done.

**Treatment and staining of cultured MoDCs:** MoDCs were seeded in 24 well plates (~3x10<sup>5</sup> cells per sample) and treated with the indicated substance. Each experiment also includes a positive control (LPS) and solvent control. 24 h after chemical treatment cell samples were divided in to halves. For one half surface staining was performed using Anti-CD86 (BD Pharmingen) whereas the second half was used for measuring cytotoxicity by 7-AAD (7-Amino-Actinomycin D) staining.

**Flow cytometric analysis:** Marker expression and 7-AAD staining were measured using a Coulter Epics XL flow cytometer (Beckman Coulter). Data were analysed using EXPO32 software.

## Results

Monocytes differentiate to immature dendritic cells after supplementing the media with rhIL4 and rhGM-CSF

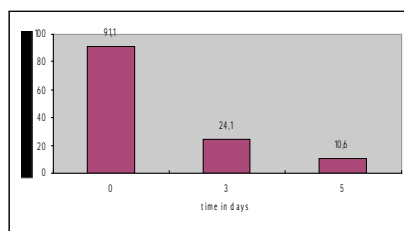


Fig. 1 Percentage of CD14<sup>+</sup> cells during incubation with rhIL4 and rhGM-CSF. Most cells lose the monocyte marker during a 5 day incubation period. The results of two independent experiments are shown.

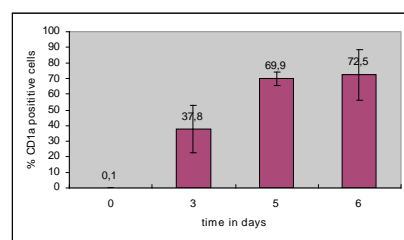


Fig. 2 Percentage of CD1a<sup>+</sup> cells during incubation with rhIL4 and rhGM-CSF. While most cell lost their monocyte marker (Fig.1) they became positive for CD1a (DC marker) during the incubation period. The results of three independent experiments are shown.

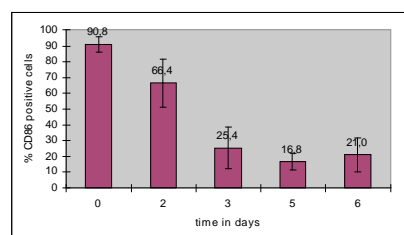


Fig. 3 Percentage of CD86<sup>+</sup> cells during incubation with rhIL4 and rhGM-CSF. Basal expression of CD86 in monocytes was high (>90% positive cells!). However the percentage of CD86 positive cells decreased during the first three days of incubation remarkably. The results of six different experiments are shown.

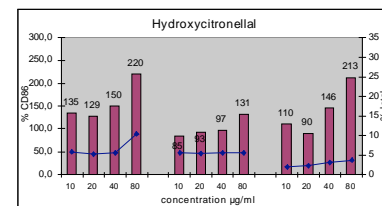


Fig. 4 Donor variability resulting in MoDCs with different abilities to respond to allergens (also irritants, data not shown). Three independent experiment with MoDCs from three different donors are presented.

## In vitro prediction of skin sensitization

Chemical	Potency Category (LLNA)	MoDC	+ / total exp.
Oxazolone	extreme	-	0/3
Hydroquinone	extreme	+	3/3
Propyl gallate	strong	+	2/3
Isoeugenol	moderate	+	2/3
Sodium lauryl sulphate	moderate	+ / -	10/21
4-Chloraniline	moderate	-	0/4
α-Hexyl cinnamic aldehyde	moderate	+	3/3
α-Amyl cinnamic aldehyde	weak	+	4/4
Eugenol	weak	+	3/3
Hydroxycitronellal	weak	+	5/6
Lactic acid	NS	-	0/3
Benzalkonium chloride	NS	-	0/3
p-amino benzoic acid	NS	-	0/4

Tab. 1 Results of the MoDC assay in comparison to in vivo data (Local Lymph Node Assay). For each chemical at least three independent experiments were done. Within one experiment at least three doses of each chemical were tested (the highest dose was slightly cytotoxic). A substance was regarded as in vitro positive, if 150% of the cells in comparison to the solvent control were CD86 positive (one test concentration was sufficient) and the cytotoxicity was below 15%. Green fields: in vitro and in vivo assay with same results. Red fields: in vitro false negatives.

## Conclusion

- MoDC-Assays could be powerful tools in a test battery using several in vitro generated key information for predicting skin sensitization (eg bioavailability and peptide reactivity).
- Due to donor variabilities MoDCs of several donors (at least three) have to be used.
- 4-Chloraniline and Oxazolone are false negatives. Because of this limited sensitivity MoDC-Assays could not be used as a stand alone assay to predict skin sensitization in vitro.
- MoDC-Assays could probably be improved if skin metabolism would be integrated.