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Dendritic cell migration assay: A potential prediction model for identification of contact allergens

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ABSTRACT

This manuscript describes methodology and a prediction model for the MUTZ-LC migration assay. The assay represents the physiological change in Langerhans cell (LC) behavior after exposure to a sensitizing chemical, resulting in LC migration from the epidermis to the dermis. MUTZ-LC are derived from the commercially available MUTZ-3 cell line. Upon exposure to a sensitizer MUTZ-LC migrate preferentially towards CXCL12 whereas upon exposure to a non-sensitizer MUTZ-LC migrate towards CCL5. A CXCL12/CCL5 ratio >1.10 in 2/3 independent experiments is indicative of a sensitizer, whereas a CXCL12/CCL5 ratio $\leqslant$1.10 is indicative of a non-sensitizer. At non cytotoxic chemical concentrations 9 sensitizers (2,4-dinitrochlorobenzene, paraphenylendiamine, cinnamaldehyde, isoeugenol, nickel-sulfate, tetramethylthiuram disulfide, eugenol, cinnamic-alcohol, ammonium-hexachloroplatinate) were distinguished from 4 non sensitizers (sodium lauryl sulfate, salicylic acid, phenol, octanoic acid). Critical points in assay performance are (i) MUTZ-3 passage number after thawing (p6–p40); (ii) cell viability (>80%); (iii) standard curve to optimize correlation of fluorescence with cell number; and (iv) optimization of the concentration of rhCXCL12 and rhCCL5 in transwell. The protocol has been tested in three European laboratories and results suggest that it may provide working conditions for performing the DC migration assay which is aimed at distinguishing sensitizers from non sensitizers.

1. Introduction

The aim of the Sens-it-iv EU funded project, (#018681) was to develop and optimize a battery of *in vitro* assays which when used together in a tiered strategy would be capable of predicting the potential of a chemical to induce sensitization in a human. Once validated, these assays may replace the Local Lymph Node Assay for risk assessment of potentially sensitizing substances (Kimber et al., 1990, 1991). During the development of the assays the following mechanistic steps were taken into account: (i) In order for a potential sensitizer to cause an allergic reaction it must first penetrate or damage the stratum corneum to exert its effect on the viable epidermal and dermal layers below. (ii) Once a chemical has penetrated the stratum corneum, it will trigger keratinocytes to release alarm signals in the form of cytokines and chemokines. In parallel, the chemical pro-hapten, needs metabolic activation in order to become protein reactive. (iii) The Langerhans Cells (LCs) within the epidermis will then become activated by the hapten-protein complex and undergo maturation and differentiate from antigen-capture and processing cells into potent immunostimulatory dendritic cells (DCs) which are able to present antigen effectively to effector T-cells. (iv) The LCs migrate from the epidermis, to the dermis and then onto the paracortical area of the regional lymph nodes, where they present the allergenic epitope to naïve T-cells (Aiba et al., 1997; Lanzavecchia and Sallusto, 2001; Nosbaum et al., 2009; Reid et al., 2000; Saint-Mezard et al., 2004). (v) This results in expansion and differentiation of allergen reactive T cells, thereby forming specific effector and memory T cells, which migrate via the efferent lymphatics into the bloodstream and recirculate through the body (Sallusto et al., 1999). The individual is then sensitized to the chemical and will develop an allergic contact dermatitis when exposed again to the same chemical.

Five assays were identified which reflect the different steps involved in sensitization: (i) An epidermal equivalent potency assay for determining sensitizer potency (dos Santos et al., 2011; Spiekstra et al., 2009); (ii) A keratinocyte cell line (NCTC) based assay in which increased production of intracellular IL-18 may distinguish

Abbreviations: MUTZ-LC, MUTZ-3 derived Langerhans Cells; CMC, cytokine maturation cocktail.

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skin sensitizers from respiratory sensitizers and non sensitizers (Corsini et al., 2009; Galbiati et al., 2011); (iii) A genomic biosignature using the human MUTZ-3 cell line (GARD) which may identify sensitizers and determine sensitizer potency (Johansson et al., 2011); (iv) A MUTZ-3 derived Langerhans Cell (MUTZ-LC) migration assay which may distinguish sensitizers from non sensitizers based on differential chemokine receptor and migratory properties of MUTZ-LC after sensitizer and non sensitizer exposure (Rees et al., 2011; Ouwehand et al., 2010b); and (v) T cell assays based on T cell proliferation using radioactive labeling or dilution fluorochromes such as carboxyfluorescein succinimidyl ester (CFSE) and also T cell activation markers, such as CD137, CD154 (CD40L), and IFN-γ to identify antigen-activated T cells (Martin et al., 2010). This manuscript describes in detail the current methodology of a prediction model for the MUTZ-LC migration assay. This assay represents the functional migration of LC from the epidermis to the dermis after exposure to a sensitizing chemical.

Migration of LC is associated with an increase in CXCR4 and a decrease in CCR1/CCR2/CCR5 receptors on the maturing LC's combined with an increase in the secretion of the chemokine CXCL12 (ligand for CXCR4) from fibroblasts in the dermis (Ouwehand et al., 2008) (Fig. 1). The increase in CXCL12 secretion by fibroblasts is a general stress signal since it is induced by TNF-α (Ouwehand et al., 2008). The mature LC's eventually travel in a CXCR4/CCR7 dependent manner to the lymph nodes where they may prime T cells resulting in sensitization (Villablanca and Mora, 2008). In contrast to sensitizer mediated LC migration via the CXCR4/CXCL12 axis, non sensitizer (irritant) mediated LC migration is mediated by maintained CCR1/CCR2/CCR5 expression and low CXCR4 expression on immature LC together with upregulated CCL5 secretion by dermal fibroblasts. Increased levels of CCL5 result in drawing CCR1/CCR2/CCR5 expressing LC from the epidermis into the dermis (Ouwehand et al., 2010a). The DC migration assay is based on the differential chemokine receptor expression on LC after exposure to sensitizers (CXCR4) or non-sensitizers (CCR1, CCR2 and/or CCR5) and their ability therefore to migrate preferentially to CXCL12 or CCL5 respectively. This assay uses MUTZ-LC derived from the MUTZ-3 cell line (CD34+ human acute myeloid leukaemia cell line). MUTZ-3 have been shown to differentiate in a cytokine dependent manner into LC-like cells, expressing Langerin and Birbeck granules, which very closely represent the native counterpart (Masterson et al., 2002; Santegoets et al., 2008). Furthermore, mature MUTZ-LC have been shown to migrate preferentially towards CXCL12 whereas immature MUTZ-LC migrate preferentially towards CCL5 in a similar manner to native LC (Ouwehand et al., 2008, 2010a, 2010b). In the migration assay, in parallel wells of a transwell plate either human recombinant CXCL12 or CCL5 are placed in the lower chamber. MUTZ-LC, pre-exposed to non-cytotoxic concentrations of chemicals, are placed in the upper chamber of both CXCL12 and CCL5 containing wells. MUTZ-LC exposed to sensitizers (including pro-haptens) in the upper chamber of a transwell migrate preferentially towards CXCL12 in the lower transwell chamber, whereas MUTZ-LC, pre-exposed to non sensitizers in the upper chamber of a transwell migrate preferentially towards CCL5 in the lower transwell chamber (Ouwehand et al., 2010b; Rees et al., 2011). The read-out of the assay is the relative number of fluorescent MUTZ-LC entering the lower chamber expressed as a ratio of CXCL12: CCL5. A ratio >1.1 in 2/3 indepependent experiments indicates that the chemical is a sensitizer whereas a ratio $\leqslant$1.1 indicates that the chemical is a non sensitizer. This assay has been tested in a ring-study, whereby three different European laboratories (VU University Medical Centre, Amsterdam, The Netherlands; Università degli Studi di Milano, Italy; and University of West England, Bristol, UK) have shown that the assay is transferable. Intra-laboratory and inter-laboratory variation with regards to MUTZ-3 progenitor cell culture, differentiation into MUTZ-LC, maturation

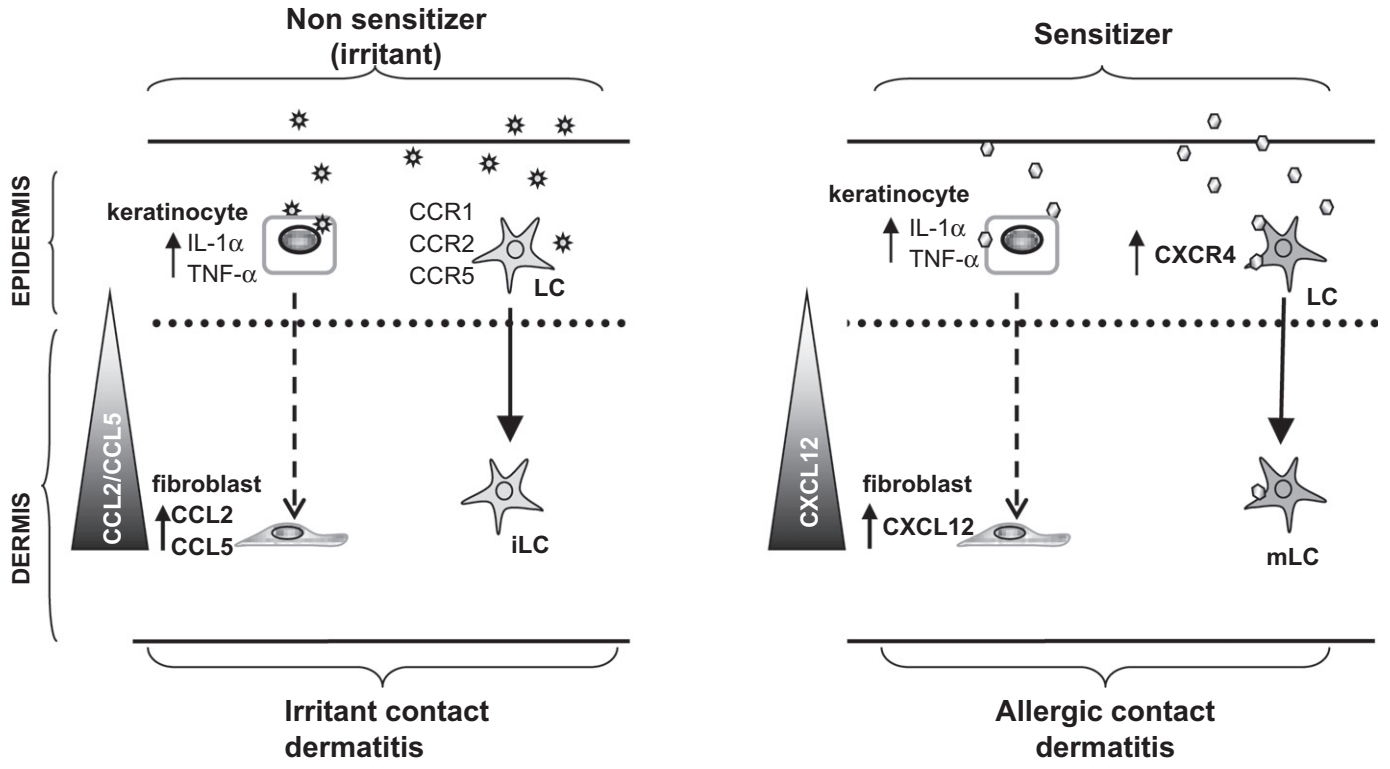

Fig. 1. Schematic overview of mechanism of LC migration in irritant and allergic contact dermatitis. The mechanism of LC migration is depicted. Large arrows pointing down indicate direction of LC migration; dotted arrows indicate cytokine conditioning; small upward arrows indicate upregulation of indicated molecules; shaded pyramides indicate chemokine gradients.