

Abstract

The sensitizing potential of chemicals is usually identified via animal studies, such as the local lymph node assay. Due to the increasing public and political concerns regarding the use of animal for the screening of new chemicals, the Colipa Skin Tolerance Task Force collaborates with and/or funds academic research groups to increase and apply our understanding of the molecular and cellular events occurring during the acquisition of skin sensitization. Fundamental and applied research is being funded in the following key areas: chemistry/peptide binding, skin metabolism, skin bioavailability, evaluation of biomarkers for DC activation and T cell proliferation. Knowledge gained from this research is being used to support the development and evaluation of novel alternative approaches for the identification and characterization of skin sensitizing chemicals. At present three non-animal test methods [Direct Peptide Reactivity Assay (DPRA), Myeloid U937 Skin Sensitization Test (MUSST) and human Cell Line Activation Test (hCLAT)] have been evaluated via interlaboratory ring trials for their potential to predict skin sensitization and were recently accepted by the ECVAM for formal pre-validation. Data from all three test methods will be used to support the development of testing strategy approaches for skin sensitizer potency prediction. The replacement of the need for animal testing for skin sensitization risk assessment is viewed as ultimately achievable and the next couple of years should set the timeline for this achievement.

Introduction

Skin sensitization is the induction of an allergic immune response following skin exposure that can be induced by a subset of chemicals. Allergic contact dermatitis is the clinical condition resulting from skin sensitization which is a delayed-type hypersensitivity reaction induced by small reactive chemicals (haptens). Guinea pig models were historically used to identify whether a chemical has the potential to induce skin sensitization in humans. More recently, a refined and reduced method, the murine local lymph node assay (LLNA) has been employed and the sensitizer potency information generated can be used to predict a safe level of human exposure (using a Quantitative Risk Assessment approach). There are, however, increasing public and political concerns regarding the use of animal testing for the screening of new chemicals. Consequently, the development of *in vitro*, *in chemico* or *in silico* models for predicting the sensitizing potential of new chemicals is receiving widespread interest. These tests will need to resume the complex interactions of a chemical with the different compartments of the immune system: The chemical must penetrate the skin and react with endogenous proteins. Some chemicals, termed prohaptens, require activation through skin metabolism in order to become haptens capable of binding to skin proteins. Haptenated carrier-proteins are internalized and processed by immature dendritic cells (DCs) that become activated. The activated DCs start to migrate from the epidermis into the draining lymph node, complete maturation and present fragments of the haptenated carrier-proteins to T-helper cells, resulting in an antigen-specific immune response.

Colipa, the European trade association representing the interests of the cosmetic, toiletry and perfumery industry consists of 25 national associations (representing over 2000 small and medium enterprises), 19 major international companies, 4 supporting associations and 3 corresponding members currently collaborates with several academic and industrial research groups to expand our understanding of the molecular and cellular events occurring during the acquisition of skin sensitization. At present fundamental and applied research is being funded in multiple key areas, such as modeling of skin bioavailability, hapten chemistry, peptide binding, skin metabolism, dendritic cell activation and T cell proliferation.

Portfolio of research projects

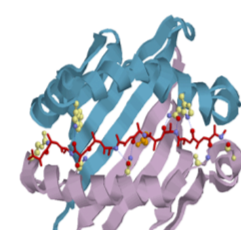
Skin exposure & bioavailability



***In silico* mathematical model for calculating the epidermal bioavailability of contact allergens**

G.Kasting, *Univ. Cincinnati*, F.Gerberick, P. Kern, *P&G*

Modification of skin proteins by chemical sensitizers



Direct Peptide Reactivity Assay (DPRA): Next generation assay

J.P. Lepoittevin, *Univ. Louis Pasteur, Strasbourg, France*; G.F. Gerberick, *P&G*

Evaluation of the sensitizing potential of chemicals based on their chemical reactivity

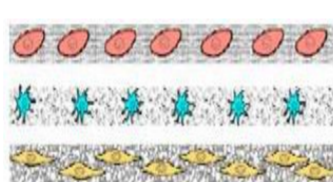
M. Pallardy, *Univ. Paris-Sud, France*; JP. Lepoittevin, *Univ. of Strasbourg, France*



Characterization of the metabolic capacity of human skin & current *in vitro* model systems

E. Fritsche, *Univ. Düsseldorf, Germany*; R. Edwards, Z. Zhu, *Imperial College London, UK*

Skin Innate Immune response



Construction of an immunocompetent three dimensional human skin model

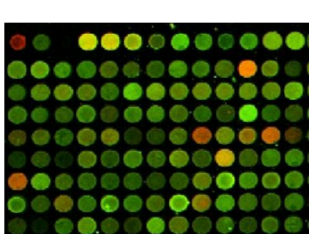
A.G. Maghami, *Univ. of Nottingham, UK*; J. Haycock, *Univ. of Sheffield, UK*

Sensitizer-induced DC activation



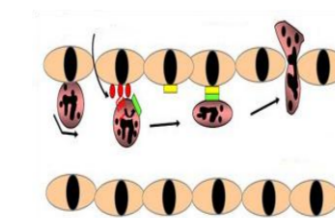
Implication of signal transduction pathways in DC maturation, a potential predictive model for allergic contact dermatitis.

M. Serres, *INSERM Lyon, France*; M. Tailhardat, *LVMH, France*



Gene expression changes predicting DC activation potential

C. A. Ryan, L. A. Gildea, L. Foertsch, G. F. Gerberick and C. Goebel, *P&G*



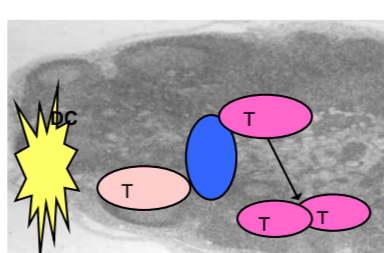
Multi-parameter dendritic cell biosensor system to identify contact allergens

A. Takashima, *University of Toledo Ohio, USA*

Identification and exploitation of molecular mechanisms underlying the homing of DCs to lymph nodes

J. E. Pease, *Imperial College London, UK*

T cell priming assays



Development of T cell priming assays using human PBLs depleted in CD25+ Treg cells

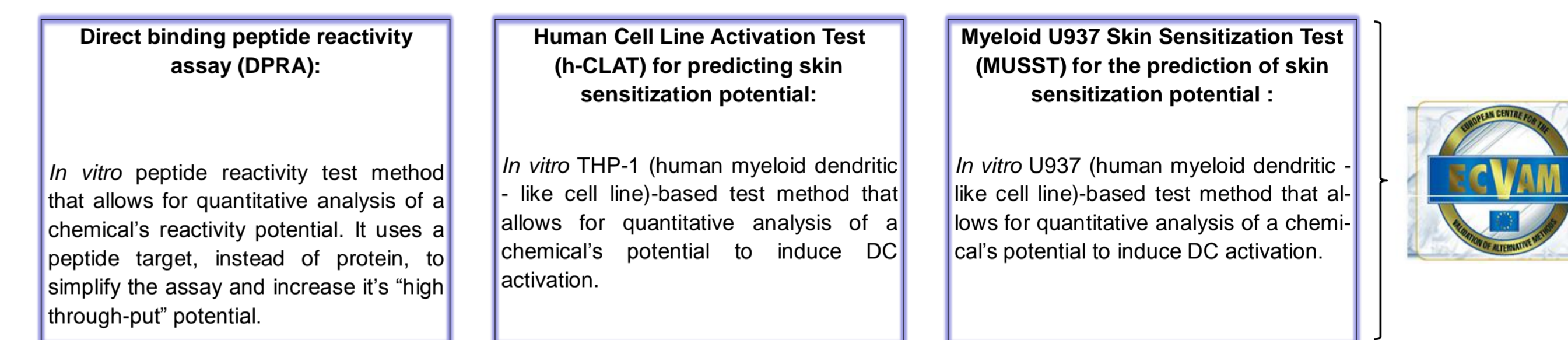
M. Vocanson and J. F. Nicolas, *INSERM Lyon, France*; M. Tailhardat, *LVMH, France*

Correlation between the strength of contact allergens, effector and regulatory T cell frequency and T cell receptor repertoire

S. Martin and T. Jakob, *University Medical Center Freiburg, Germany*

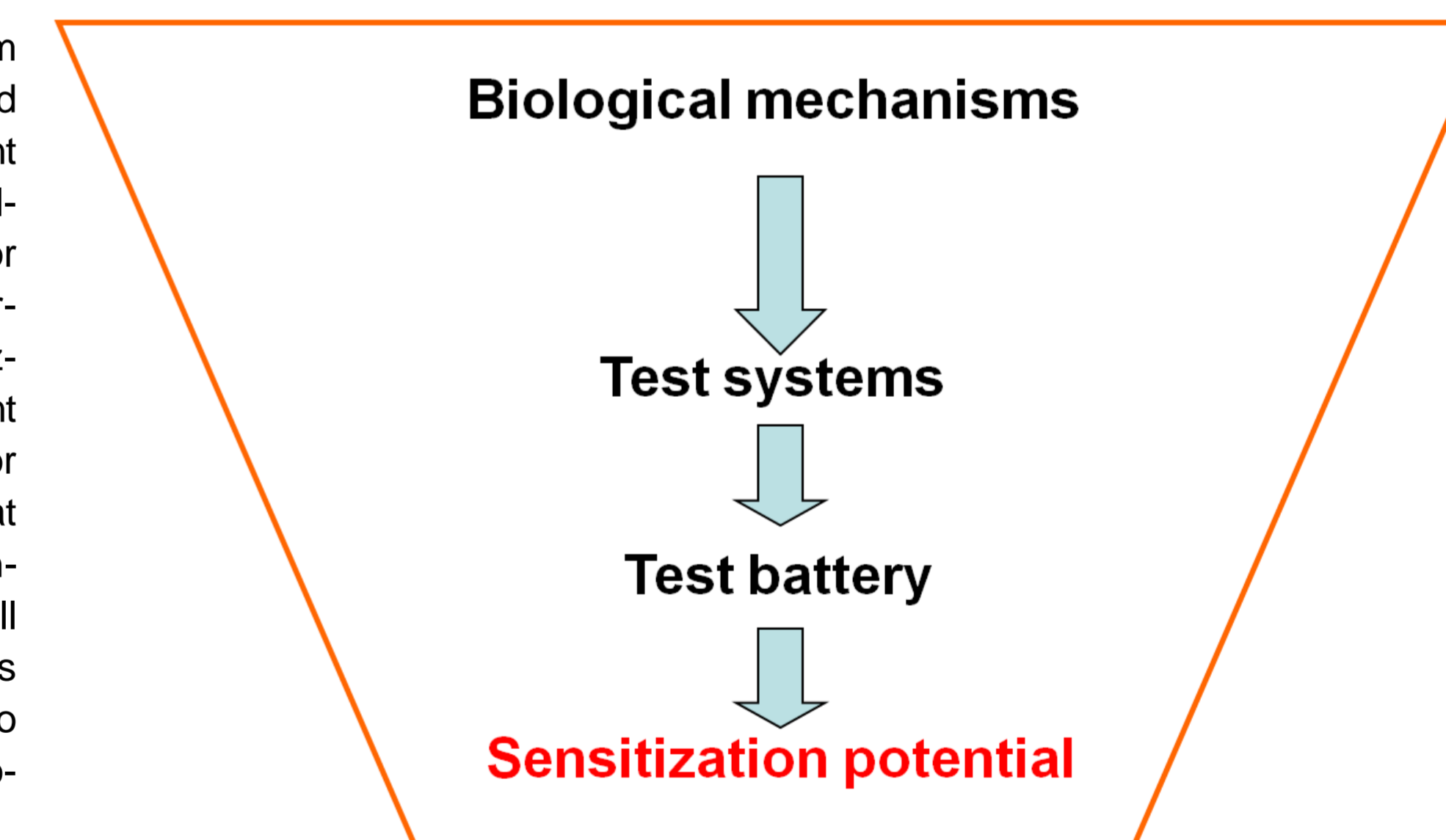
Method pre-validation activities

At present one *in chemico* (direct peptide reactivity assay (DPRA)) and two *in vitro* test methods (cell based assays (MUSST) and (hCLAT)) have been evaluated for their potential to predict skin sensitization potential within Colipa interlaboratory ring trials and accepted by the European Centre for the Validation of Alternative Methods (ECVAM) for phase III pre-validation.



Integrated testing strategy

Knowledge gained from this research is being used to support the development and evaluation of novel alternative approaches for the identification and characterization of skin sensitizing chemicals. The current replacement rationale for skin sensitization is that data from several non-animal test methods will need to be combined as part of a testing strategy to predict skin sensitizer potency information.



Conclusions

The range of research projects supported by the Colipa Skin Tolerance Task Force exploits most of our current understanding of the biological events occurring during the acquisition of skin sensitization. It is expected that the knowledge gained by this global research effort and the synergies that should appear will allow the development of novel *in vitro* approaches for the identification and characterization of skin sensitizing chemicals. Moreover, the overall strategic goal of this program is to develop a battery of *in silico* / *in vitro* predictive assays that could be used in concert to identify and potentially quantify the potential of a novel chemical to induce skin sensitization in man. In this way we aim to generate data to support skin sensitization consumer safety risk assessment decisions in the absence of animal testing.