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Human T cell priming assay (hTCPA) for the identification of contact allergens based on naive T cells and DC – IFN-γ and TNF-α readout

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ABSTRACT

Many small protein reactive organic and inorganic chemicals can cause allergic contact dermatitis, a T cell mediated inflammatory skin disease. In vitro alternatives to animal testing are needed for the identification of chemicals that pose such risks to human health. We here publish the standard operation procedure for a human T cell priming assay developed primarily for the identification of contact allergens within the integrated EU project Sens-it-iv. This multiparametric flow cytometry based assay identifies chemical specific T cells based on their frequency and antigen-specific production of the cytokines IFN-γ and TNF-α at the single cell level. Using sorted naïve T cells and monocyte-derived dendritic cells pulsed with the test chemical or with chemical-protein conjugates, the successful priming of an antigen-specific T cell response is controlled after antigen-specific restimulation by cytokine readout. As the most specific response of the immune system to contact allergens the analysis of the chemical-specific T cell response may be a useful in vitro assay for hazard identification in immunotoxicology. This assay may be a valuable, highly specific element of an integrated testing strategy for the identification of chemicals and drugs that cause T cell mediated respiratory or gastrointestinal tract hypersensitivities or adverse drug reactions.
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1. Introduction

A large number of natural and synthetic chemicals can pose serious risks to human health. They come in contact with barrier surfaces such as skin, lung and gut. Some chemicals are reactive to proteins and other biomolecules, others are bioactivated by oxidation or enzymatic processes. Problems arise due to the potential of many chemicals to induce adverse reactions such as hypersensitivity, allergy and autoimmunity (Kadow et al., 2009). The use of chemicals in consumer products therefore necessitates a strict hazard identification and risk assessment strategy to warrant consumer safety. Chemical cosmetic ingredients have previously been tested in the mouse local lymph node assay (LLNA, OECD test guideline 429) (Gerberick et al., 2007) for their potential to induce allergic contact dermatitis (ACD), a T cell mediated inflammatory skin disease. Due to the 7th amendment to the EU Cosmetics Directive this assay was prohibited for cosmetic ingredients in March 2009. This has enhanced ongoing activities to replace animal testing in this area of immunotoxicology (Martin et al., 2010, 2011a; Peiser et al., 2012). However, the establishment of in vitro alternatives to animal testing is not only forced by legislation but is an immediate consequence resulting from discoveries in animal models of disease and human studies and is in the interest of scientific progress. The challenge that we are facing is to develop in vitro assays that reproduce crucial mechanisms of the highly complex in vivo disease pathogenesis. For chemical induced ACD this means that the assays which must replace the LLNA should reflect the crucial steps of the sensitization process induced in the skin and lymph nodes by contact allergens. Thus, the penetration of chemicals into the skin, their binding to extracellular and cellular proteins, the resulting innate inflammatory response and the activation and migration of dendritic cells (DC) from the skin to the draining local

Abbreviations: ACD, allergic contact dermatitis; APC, antigen presenting cell; DC, dendritic cell; DNBS, 2,4-dinitrobenzenesulfonic acid; EC3, effective concentration for a stimulation index of 3 in lymph node cell proliferation; EU, European Union; HSA, human serum albumin; hTCPA, human T cell priming assay; IFN-γ, interferon gamma; LLNA, local lymph node assay; MHC, major histocompatibility complex; MoDC, monocyte-derived dendritic cells; OECD, Organisation for Economic Co-operation and Development; SDS, sodium dodecyl sulfate; TCR, T cell receptor; TNBS, 2,4,6-trinitrobenzenesulfonic acid; TNF-α, tumor necrosis factor alpha.

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lymph nodes and their priming of naive chemical specific T cells are checkpoints that determine the potential of a chemical to eventually cause ACD (Kimber et al., 2011). It is obvious that a single assay will not be able to cover all of these aspects. Therefore, a combination of suitable assays will be used in an integrated testing strategy (Jowsey et al., 2006). Besides yes and no decisions regarding health risks, assays are urgently needed that allow for potency assessment of identified contact allergens. This feature is one of the strengths of the LLNA which produces so-called EC3 values based on the concentration of the test substance needed to induce a stimulation index of three. EC3 values can be used for the classification of contact allergens based on their potency to induce lymph node cell proliferation (Loveless et al., 2010).

While most assays in development or in prevalidation/validation processes analyse the rather unspecific innate immune responses to contact allergens (Martin, 2012) that result in skin inflammation the most specific immune response to chemical allergens is the T cell response. The modification of skin proteins by contact allergens does not only trigger innate immune responses (Martin et al., 2011b; Martin, 2012) but also provides T cell antigens in the form of haptenated proteins that are processed by antigen presenting cells (APC) (Martin et al., 2010). As a result, hapten-modified peptides are presented on MHC molecules on the surface of activated DC to naive hapten-specific T cells. Alternatively, direct modification of MHC bound peptides on the surface of APC or complex formation with such peptides and/or MHC molecules in the case of metal ions also produces T cell epitopes. Specific T cells then become activated, proliferate intensely and differentiate to effector and memory T cells that acquire imprinting signals from the skin derived DC leading to the expression of a skin specific homing receptor profile (Dudda et al., 2004, 2005). The priming of a specific T cell response concludes the sensitization phase. The T cells then migrate to the inflamed skin from the blood circulation and induce the clinical symptoms of ACD in the elicitation phase. Based on the exquisite antigen specificity of T cells and their crucial role as the main effector cells of ACD, efforts to develop T cell based in vitro assays for contact allergen identification have a long tradition (Martin et al., 2010, 2011a). The recent technological advances and the increase in knowledge regarding contact allergen function and recognition by the innate (Martin et al., 2011b; Martin, 2012) and adaptive immune system (Vocanson et al., 2009) has prompted the development of new strategies to use T cells as tools in immunotoxicology.

Until now methods for the identification of naïve T cells with a defined antigen specificity in humans are not generally established. To address the question whether a given chemical compound induces T lymphocyte responses we have developed an in vitro human T cell priming assay (hTCPA). The challenges in developing such a prediction assay are the estimated low frequency of naïve T cells with specificity for a given chemical in the peripheral blood, the potentially high activation threshold of these T cells, and the delivery of chemicals to the cell culture under conditions that prevent toxicity and allow chemical reactivity. To overcome these limitations we have developed a protocol using human DC as competent APC, a defined naïve T cell population, optimized cell culture conditions for antigen specific activation and expansion of naive T cells, and methods for detection of low frequent antigen-reactive T cells. The assay allows the identification of reactive $CD4^+$ as well as $CD8^+$ T cells already 10 days after their primary activation and includes a secondary antigen-specific restimulation step that proves the antigen-specificity of the T cell response. The great advantage of the hTCPA is its exquisite antigen specificity. Limitations are the need for primary cells and the current assay format that does not allow high throughput analyses. However, the assay is of great interest for the testing of chemicals within an integrated testing strategy since it is the only assay that addresses the final and crucial step in the sensitization process that decides whether a chemical may induce T cell dependent ACD. We are currently testing whether the hTCPA can also be used for potency assessment, a crucial feature of the LLNA not faithfully reproduced by any of the in vitro assays developed up to now (Aeby et al., 2010; Kimber et al., 2012).

The current human T cell priming assays (hTCPA) developed within and outside of Sens-it-iv primarily for contact allergen identification will certainly also be of use for the identification of respiratory sensitizers and drugs that cause T cell mediated hypersensitivities (Faulkner et al., 2012). We here present the standard operation procedure (SOP) of the human T cell priming assay (hTCPA) as developed in the integrated project Sens-it-iv (http://www.sens-it-iv.eu) funded by the EU from October 2005 to March 2011 (Dietz et al., 2010). We discuss variations of the SOP and give an outlook on future refinements of the hTCPA and its potential use for potency assessment.

2. Purpose

The hTCPA reflects the final step of the successful sensitization to a chemical allergen: the induction of a chemical specific T cell response. This assay should clearly predict a health risk for a given chemical regarding induction of ACD. Without the induction of a T cell response, ACD does not occur. The hTCPA complements assays that address sensitizing potential based on chemical action on e.g. DC and keratinocytes, i.e. chemical induced innate immune responses. It should be useful as an important and highly specific element of a tiered integrated testing strategy and should provide definite proof for the allergenic potential of a chemical.

Furthermore, the hTCPA can be used as a second or third line screening method for chemicals, which yield unclear results and for confirmation of positive results in other sensitization assays. Thus, false negative and false positive results may be significantly reduced (e.g. SDS).

3. Limitations

- Complex formation with or covalent coupling of chemicals to APC/proteins/peptides is necessary to provide immunogenic T cell receptor reactive hapten epitopes
- a reasonable frequency of T cells with chemical specific T cell receptors is required
- variability of T cell responses (T cell repertoire) between blood donors are observed

4. Method outline

Naïve T cells and chemical-modified/-pulsed monocyte-derived DC (MoDC) are co-cultured in the presence of feeder cells, costimulatory CD28 antibody, and cytokines. After 10 days IFN-γ and TNF-α production by T cells is detected after a rechallenge with chemical-modified/-pulsed MoDC.

5. Definitions/abbreviations

PBMC	peripheral blood mononuclear cells
APC	antigen presenting cell
MoDC	monocyte-derived dendritic cell
IL	interleukin
GM-CSF	granulocyte macrophage colony-stimulating factor
TNF-α	tumor necrosis factor-alpha
IFN-γ	interferon-gamma

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