by Robert Holtz

In vitro Sensitization Models: Current Progress

An Internet search of the term *skin sensitization* reveals its definition as:

> *"A condition that is 'induced when a susceptible individual is exposed topically to (an) inducing chemical allergen. The chemical allergen provokes a cutaneous immune response which, if of the required magnitude and quality, will result in the development of contact sensitization' and a subsequent exposure to the material will lead to allergic contact dermatitis (ACD)."*[1]

While it is relatively easy to find the definition of this term, it has been difficult to elucidate the cellular and molecular mechanisms by which the skin can become sensitized to certain materials, and even more difficult to develop in vitro tests to predict which materials have the potential to be sensitizing agents.

Since cosmetic and personal care product manufacturers have an obligation to ensure their products are not harmful to consumers, it is essential for them to test the skin sensitization potential of the ingredients used in their products. Presently, only animal-based methods are available for testing sensitization potential; however, the industry is emphasizing the need for animal-based method replacements. In response to this need, several interesting and promising approaches to in vitro skin sensitization testing are in development. Perhaps it will not be long before they are deemed valid and reliable alternatives to animal testing.

General Mechanism

The process of skin sensitization can be broken down into two phases. The first phase is the actual sensitization process. It begins with the application of the sensitizing agent to the skin. As the sensitizing agent penetrates the outer layers of the skin, it must covalently react with proteins found in the skin.

This covalent attachment to proteins is essential for the next step, which is the recognition and interaction of the sensitizing agent-protein complex with Langerhans cells. Langerhans cells are a type of dendritic cell and act as antigen-presenting cells in immune responses. When Langerhans cells are exposed to the sensitizing agent-protein complex, the complex is internalized by the Langerhans cell, processed within the cell, and then the complex is attached to major histocompatibility complex (MHC) proteins and re-expressed on the cell surface. The Langerhans cells then migrate from the skin to the nearest draining lymph nodes. Once in the lymph nodes, the sensitizing agent-protein complex, now bound to the MHC proteins on the surface of the Langerhans cells, is introduced to and thus activates T-lymphocytes.

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In response to this activation, T-lymphocytes that specifically recognize the sensitizing agent will proliferate and stand ready to respond to additional exposure to the sensitizing agent. The response to a subsequent exposure to the sensitizing agent represents the second phase in skin sensitization: the elicitation process. In this phase, the now activated T-lymphocytes will release cytokines and chemokines, which attract other immune cells to the site and provoke the cutaneous inflammation response that is associated with ACD in the location of the skin where the sensitizing agent was reapplied.

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Cell-based Approaches

Since Langerhans cells play a vital role in the sensitization process, they are an appealing target for developing an in vitro model to predict the sensitization potential of a test material. However, Langerhans cells make up only a small percentage of epidermal cells and it has proven difficult to recover them in sufficient quantities for use in testing, and to recover them for in vitro culture use in such a way that they behave the same way as they do in vivo.

As an alternative, many approaches have generated Langerhans-like cells from other cell types. These cell types mainly include hematopoeitic cells isolated from neonatal cord blood or bone marrow and peripheral blood mononuclear cells (PBMCs). These cells are commonly marked with a specific cell surface marker called CD34, which is a protein that functions in cell-to-cell adhesion, and are hence referred to as CD34+ cells. When stimulated with the proper cytokines, CD34+ cells can become dendritic cells that are very similar to Langerhans cells.[2]

Current sensitization testing approaches using either Langerhans cells proper, or Langerhans-like cells, involve culturing the cells with known sensitizing agents, known nonsensitizing agents or known irritating agents, and

then measuring the biological responses of the cells. When Langerhans cells internalize the sensitizing agent-protein complex, this can initiate a complex set of changes within the cell. These changes include differential expression of cell surface markers, the activation of various intracellular signaling pathways, and the release of diverse cytokines.

While these measurements of biological responses hold promise, there have been some difficulties with this approach. For example: some of the cytokines that are released in response to sensitizing agents are also released in response to irritating agents;[3] different sensitizing agents induce the expression of different cell surface markers when cells from different donors are used, making it difficult to find a common end point for all potential sensitizing agents;[4] or the cytokine used as a measurement is not sensitive enough to discriminate between sensitizing agents of differing potency.[5]

Recent work by Gildea et al.[6] attempted to address the difficulties described above. This group used PBMC dendritic cells obtained from multiple donors, and also used various concentrations of a wide variety of sensitizing agents and irritating agents. After incubating the cells with these agents, genomic changes were determined using DNA microarrays and these changes were confirmed using RT-PCR based methods. The results from this study identified a set of genes that could discriminate sensitizing chemicals from both nonsensitizing chemicals and irritating chemicals in all donors. These gene markers included NOTCH3, ARHGDIB, CCL23, CD1E, CYP27A1, HML2 and S100A4 (see **Gene Markers**).

While the exact role of some of these gene markers in the sensitization process is not clearly defined, if confirmed, this set of gene markers could be remarkably useful in in vitro models for predicting the sensitization potential of cosmetic ingredients.

Peptide-based Approaches

Before interacting with Langerhans cells, the sensitizing agent must bind to a skin protein. Sensitizing agents are too small on their own to provoke an immune response and so they need to be bound to the larger protein molecules in order to interact with the Langerhans cells. In addition, most of the known sensitizing agents either have electrophilic properties or can undergo biotransormation reactions in the skin to convert them to a metabolite that has electrophilic properties.[7]

Electrophiles have either a partial positive charge or a full positive charge and are attracted to negatively charged, electron-dense regions in other molecules or ions. Proteins, on the other hand, have amino acids with side chains that are nucleophilic.

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Nucleophiles have either a slightly negative charge or full negative charge, and are highly attracted to regions with a positive charge in other molecules. With skin sensitization, the electrophilic sensitizing agent can attack the nucleophilic amino acid side chains, resulting in the formation of either a covalent bond or a co-ordination bond if the sensitizing agent is a metal.[8] It is after this protein binding that the sensitizing agent can react with the Langerhans cells and initiate the immune response.

Of the 22 amino acids, recent work by Gerberick et al.[7] has shown that three have side chains that are probable targets for sensitizing agents and could be used as targets in an in vitro-based peptide-based assay to screen sensitizing agents.

For the peptide-based assay, the three amino acids—lysine, cysteine and histidine—were incorporated into a synthetic peptide and reacted with various sensitizing agents and nonsensitizing agents. At the end of the reaction period, the test materials, peptides and modified peptides were separated and analyzed using HPLC. The study found a good correlation between the amount of peptide substrate that was modified and the potency of the sensitizing agent. These results suggest that an adaptation of this method for high throughput analysis could be an excellent in vitro method for screening materials for sensitization potential.

GENE MARKERS

NOTCH3: This gene encodes the notch 3 cell surface receptor normally expressed in vascular smooth muscle cells; however, recent evidence has suggested that this receptor protein also plays a vital role in regulating T-cell differentiation. [9]

ARHGDIB: This gene encodes a hematopoietic cell-oriented protein that inhibits dissociation of GDP from the rho subfamily of ras-related proteins and plays a role in immune responses.[10]

CCL23: This gene encodes the inducible cytokine A23, a CC chemokine that binds CC chemokine receptor 1 (CCR1) and induces calcium flux, inhibits proliferation of myeloid progenitor cells, and induces chemotaxis in monocytes and resting T lymphocytes.[10]

CD1E: This gene encodes a small protein that plays a role in the antigen-presenting process of dendritic cells. [11]

CYP27A1: This gene encodes an enzyme that can catalyze the hydroxylation of bile acids and also the bioactivation of vitamin D3.[12]

HML2: This gene encodes macrophage lectin 2, a calcium-dependent cell surface c-type lectin.[10]

S100A4: This gene encodes a calcium-dependent protein capable of binding myosin All to enhance cell mobility. [13]

The Future of In vitro Testing

Both cell-based and peptide-based in vitro methods for measuring the sensitization potential of test materials show excellent promise. If universal gene markers identified in the cell-based measurements are confirmed to be consistent from individual to individual, able to discriminate nonsensitizers from sensitizers, and also rank sensitizers based on potency, then it would be easy to adapt the assay to a high throughput format using ELISA-based measurements for the markers.

In addition, if the peptide-based measurements continue to show good agreement for all sensitizing agents, then they could also be converted to ELISA-based assays for high throughput analysis of materials. While both methods alone may eventually be valid alternatives to animal testing, perhaps the greatest benefit would come from using both methods.

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