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Immune functions of the human skin. Models of *in vitro* studies using Langerhans cells

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Abstract

Human skin constitutes the first immune defense barrier. Among the epidermal cells, the Langerhans cells, which belong to the dendritic cells, represent the pivotal cells in cutaneous immune reactions. The possibility of obtaining human Langerhans cells either from human skin or by *in vitro* generation from $CD34^+$ hematopoietic precursors opens the way to studies reproducing the successive steps of the Langerhans cells' role in contact dermatitis.

Abbreviations: LC, Langerhans cell

Introduction

In humans, the skin is the interface between the internal milieu and the external environment. The skin can be the target of various types of insult: physical (heat, cold, UV radiation from the sun), chemical (haptens), biological (bacteria or viruses), and psychological (stress). Human skin, together with the mucosa, constitutes the first immune defense barrier engaged in the protection of self. Among the epidermal cells, the Langerhans cells represent the pivotal cells in the cutaneous immune reactions.

The Langerhans cells (LC)

Described in 1868 by Paul Langerhans (Langerhans, 1868), LC come from cells of medullary origin that belong to the dendritic cell family (Schmitt et al., 1989). The origin of LC has been demonstrated in the mouse (Katz et al., 1979) and more recently in the human (Perreault et al., 1984). In 1992, LC were obtained *in vitro* by culture of hematopoietic $CD34^+$ progenitors with GM-CSF and TNF-α (Caux et al., 1992). $CD34^+$ cells are present in bone marrow, in cord blood and in adult circulating blood (Strunk et al., 1996). These $CD34^+$ cells are able to mature into two distinct subpopulations characterized by the expression of CD1a or CD14 (Caux et al., 1996). $CD34^+$ hematopoietic cells, which express the CLA antigen, preferentialy differentiate into LC (Strunk et al., 1997). The precursors of LC migrate from the bone marrow to the epidermis via the blood vessels. The role of the epithelial microenvironnement in the maturation of epidermal LC (formation

of Birbeck granules or strong expression of CD1a antigens) is not yet clearly established. The recent results obtained by Régnier and Staquet (Régnier et al., 1997; Staquet et al., 1997a) in the reconstruction of an epidermis containing LC open a new path in this field of research.

In normal human epidermis or mucous membrane, LC express various surface glycoproteins: HLA class I and class II molecules, receptors for the Fc component of IgG, for the C3b component (CR1) and the C3bi component (CR3), CD1, CD4 antigens and some integrins. Epidermal LC express only CD1a and CD1c. The CD1 antigens appear to be part of a receptor site since they are internalized via coated pits, coated vesicles, and endosomes, organelles characteristics of receptor-mediated endocytosis. Epidermal LC also express CD4 molecules and several surface antigens related to the integrin family. In the β_1 family, VLA_4, VLA_5 and VLA_6 are the more highly expressed. The β_2 family is present on LC membrane but β_4 and β_5 families are not found (except for v chain of the β_3 family). LC are present in the skin (the epidermal density of LC is $700/mm^2$ or $1.6 \times 10^5/mm^3$) and in all mucous membranes (oral, anal, vaginal mucosa, nasal and lung epithelia, bladder epithelium). In oral mucosa, the density of LC is $900/mm^2$. The key role of these cell populations is immune surveillance against foreign antigens. After contact with such exoantigens, they act as antigen-presenting cells through a specific cooperation with $CD4^+$ lymphocytes after migration from the peripheral epithelia to the proximal lymph nodes. The main functions of LC are antigen binding, antigen processing and migration to lymph nodes in order to present the antigens to naive T cells. Experimental studies clearly demonstrated that LC are necessary to induce an *in vivo* (or an *in vitro*) T cell proliferative response to soluble protein antigens and simple chemical haptens. Sensitization to contact allergens or foreign alloantigens depends upon the presence and normal distribution of LC within the epidermis (Dezutter-Dambuyant, 1995).

Keratinocytes and epidermal cytokines

The skin's primary function is as an organ of protection and its principal barrier is the epidermis. The epidermis is made up almost exclusively of epithelial cells called keratinocytes. These cells behave as important partners of the Langerhans cells in the immune response. Indeed, the keratinocytes are capable of producing various cytokines and growth factors. These soluble mediators can act either in an autocrine manner on the keratinocytes themselves or on the surrounding dendritic or lymphocytic cells. In addition, keratinocytes are able to react to the presence of cytokines produced by other cells in the local immune response, especially those produced by T lymphocytes (Schröder, 1995; Ullrich, 1995).

Among the cytokines secreted by keratinocytes, it is possible to identify the interleukins (IL-1, IL-3, IL-6, IL-7, IL-8, IL-10, and IL-15) (Enk et al., 1995), colony-stimulating factors (M-CSF, G-CSF, and GM-CSF), growth factors (FGF, PDGF, IGF, TGF-α and TGF-β), chemotactic factors (IL-8, IP-10, and GRO), and immunosuppressive factors (IL-10). These factors make up the soluble elements that are capable of modifying the local immune response. Under normal conditions, keratinocytes do not produce cytokines or secrete only small amounts of the cytokines, but when they are activated under the effect of chemical, physical, or endogenous aggression by various inflammatory mediators, they begin to secrete proinflammatory cytokines. The effects of these cytokines can be multiple and, in a general way, constitute a reactive balance that can be shifted by the presence of one or the other of these cytokines. Certain cytokines can induce cellular activation, others can

induce the attraction and migration of certain cells, and others can induce cell proliferation. Finally, some of the cytokines are capable of inducing the production of other cytokines, and thereby of regulating the inflammatory effects of certain others (Müller et al., 1995; Blauvelt et al., 1996).

In vitro reproduction of contact hypersensitivity

The possibility of obtaining human epidermal Langerhans cells (either from normal human skin by dermo-epidermal separation then isolation by trypsin digestion, by getting them after spontaneous migration *ex vivo* in culture plates, or by their production from $CD34^+$ medullary precursors) opens the way to *in vitro* studies reproducing the successive steps of the role of LC in contact dermatitis.

In vitro model of cell migration

Older experiments have shown *in vivo* that Langerhans cells that had taken up a fluorochrome in the skin were likely to be recovered 2–3 days later in the proximal lymph nodes owing to their identification by the fluorochrome. Other experiments have demonstrated the migration of Langerhans cells by way of the lymphatics. The demonstration of different families of adherence molecules at the surface of normal human epidermal Langerhans cells has led to experiments entirely *in vitro* that are aimed at deciphering the mechanism of migration of Langerhans cells from the skin to the lymph nodes. These cells must, in effect, cross a basement membrane that is made up essentially of laminin and of type IV collagen, and then pass through the connective tissue composed mostly of diverse types of collagen and fibronectin to reach the proximal lymph nodes.

Recent work has revealed the different mechanisms of expression and of regulation by the adhesion molecules in relation to various constituents of the extracellular matrix in the dermal connective tissue (Staquet, 1997; Staquet et al., 1997b). Modern methods using different compartments separated by a reconstituted basal membrane have likewise shown that the fixation of a hapten on the surface of Langerhans cells increases and stimulates their migratory capacity (Kobayashi et al., 1994).

Reproduction in vitro of contact hypersensitivity

Cutaneous contact hypersensitivity, a model of delayed hypersensitivity, requires two successive contacts between the dendritic cells and the antigen. The first contact leads to the formation of a clone of T memory cells that can be activated secondarily by a later contact with the same antigen presented by dendritic cells. This is the mechanism of contact eczema.

It has been possible to reproduce this reaction entirely *in vitro* with Langerhans cells and normal human lymphocytes in an autologous system, that is, one that comes from a single donor. The first step is to allow the Langerhans cells to come into contact with a hapten such as trinitrophenyl (TNP). The cells are then capable of stimulating, in a primary response, autologous T lymphocytes if the Langerhans cells were previously incubated for 2 days. This preculturing of the Langerhans cells leads to their maturation *in vitro* and makes them phenotypically and functionally comparable to interdigitating cells of the proximal lymph nodes. In the second step, lymphocytes thus stimulated are cultivated again with Langerhans cells that carry the hapten. Under these conditions, one can observe a very strong proliferative response of the lymphocytes: the secondary response. Using this model, various T lymphocyte clones specific for a hapten can also be individualized (Moulon et al., 1993). This set of steps which reproduces the human delayed contact hypersensitivity reaction *in*

vitro is the model of choice to investigate the mechanism of presentation of hapten, to try to modulate it, to suppress the capacity of Langerhans cells to present haptens and, finally, to try to estimate precisely in a predictive way the stimulating capacities of various substance destined to come into contact with the skin (Krasteva et al., 1996).

Very recently, LC obtained *in vitro* by culture of $CD34^+$ hematopoietic progenitors and autologous T lymphocytes from cord blood were used in a similar test (Rougier et al., 1998).

Conclusions

In human cutaneous biology, immunodermatology is one of the areas of research that is in full expansion. Indeed, the skin itself constitutes an extended immune organ that is both the source and the target of the immune response.

Easy access to the skin makes it a choice material for studies of cellular immune responses or, in a more general sense, of autoimmune responses. In addition, Langerhans cells constitute the best-known model of dendritic cells, the only cells capable of immunizing an individual against a substance with which it has never been in contact. For this reason, Langerhans cells constitute a remarkable model for basic immunological studies that are aimed at understanding the initial steps of the immune response that involve these dendritic cells.

The possibility of producing Langerhans cells *in vitro* has opened the way to many kinds of research dealing with their capacity to treat antigens, to migrate across connective tissues and through the lymphatics, and to cooperate with T lymphocytes, and with the mechanism of presentation of antigens to these cells. All these steps can now be examined either in animals or in *in vitro* models, by which latter means animal experiments can be avoided.

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