

Expression of PGP9.5 on Langerhans' Cells and Their Precursors

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Langerhans' cells are epidermal dendritic cells, derived from blood precursors. Their main function is antigen presentation to T-cells. They are able to express neuronal proteins, such as neuron-specific enolase or substance P-receptor. They are closely associated with nerve fibres. PGP9.5 is the most specific neuronal protein in the epidermis. Epidermal Langerhans' cells can express PGP9.5 if denervated. Using flow cytometry, we found that cultured CD34+ precursors did not express PGP9.5, whereas suspensions of fresh or cultured Langerhans' cells could express this neuronal protein. Precursors of Langerhans' cells are not able to express PGP9.5, suggesting that they are not mature enough or that the capacity to express PGP9.5 may be acquired only in the epidermis. The function of PGP9.5 on Langerhans' cells and mature dendritic cells remains unknown. PGP9.5 might be related to dendritic cell maturation or to the lack of contacts with nerve endings. Key words: dendritic cell; CD34; neuroimmunology; skin.

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Langerhans' cells (LC) (1) belong to the family of dendritic cells. As antigen-presenting cells (APC), LC exhibit an important capacity to initiate primary and secondary immune responses towards foreign proteins encountered at the epithelial interface (epidermis or mucosa) with the environment. They are recognized through the expression of CD1a antigen and specific organelles: the Birbeck granules.

LC originate from bone marrow and circulate as precursors. In bone marrow and blood, these precursors express CD34 antigen (2). Culture of precursors of LC obtained from cord blood with granulocyte/macrophage-colony stimulating factor (GM-CSF) and tumour necrosis factor α (TNF α) (3) is one of the most useful cultures for study of precursors of LC.

LC are closely associated with nerve fibres in the epidermis (4, 5) and share some properties with cells from the nervous system (6). LC and their precursors express certain proteins usually encountered in cells of the nervous system: protein S100 and neuronal specific enolase (NSE) (7, 8). S100 protein or NSE are usually not expressed by other dendritic cells or monocytes/macrophages. The function of these proteins remains unknown. LC are able to synthesize some neuromediators (derived from pro-opio-melano-cortin (POMC) (9) and to express receptors to neuromediators, such as calcitonin gene-related product (CGRP) (4), POMC-derived peptides (10), gastrin-releasing peptide (GRP) (11), substance P (12), and probably β -endorphin (13) or catecholamins (14). It is probable that LC express other neuromediators and receptors

for other neuromediators, in the same manner as monocytes and macrophages. We have recently reviewed the known interactions between LC and nervous system in the skin and we propose that these 2 cells are integrated in a neuro-immuno-cutaneous system (6).

PGP9.5 is a carboxy-terminal ubiquitin hydrolase and a highly specific neuronal marker (15). In the epidermis, LC are able to express PGP9.5 if they are denervated (16). The aim of this study was to determine whether this protein could be expressed on suspensions of LC and their precursors.

MATERIAL AND METHODS

Preparation of suspensions of Langerhans' cells

Human epidermal cell suspensions were obtained from normal skin removed during plastic surgery, as previously described (11). Briefly, the epidermis was separated from the dermis and the epidermis by incubation for 1 h with 0.05% trypsin (Difco Laboratories, Detroit, USA). Epidermal fragments were pooled in HBSS medium (Gibco Life Technologies, Cergy-Pontoise, France) supplemented with 10% foetal calf serum. Single epidermal suspensions were obtained by repeated pipetting of the epidermal sheets and filtration through sterile gauze. Purification of LC from epidermal suspensions were achieved by 3 consecutive gradient centrifugations on Lymphoprep (Flobio, Courbevoie, France) for 20 min at 400 g. For the last enrichment step, LC were layered on Lymphoprep previously diluted with distilled water. The resulting interface population routinely consisted of 70–90% LC, as confirmed by immunofluorescence staining with anti-CD1a monoclonal antibody (DMC1, INSERM U346) and flow cytometry analysis. Cell viability was assessed by trypan blue exclusion and reached 100%. LC suspensions were maintained in culture for 3 days in RPMI 1640 (Gibco, Grand Island, NY, USA) supplemented with 10% heat-inactivated foetal calf serum (Gibco), 5×10^5 M 2-mercaptoethanol (Sigma), 100 U penicillin per ml, 100 μ g streptomycin per ml.

Culture of CD34+ precursors

CD34+ cells, including precursors of LC, were obtained from cord blood, as previously described (3). Briefly, cord blood was collected according to institutional guidelines during normal full-term deliveries. Mononuclear cells were isolated by Ficoll-Hypaque gradient centrifugation (Pharmacia, Uppsala, Sweden). CD34+ cells were purified using MACS CD34 progenitor cell isolation kit (Miltenyi Biotec, Bergisch-Gladbach, Germany) according to the manufacturer's instructions. The purity and viability of the CD34+ population were respectively more than 80% and 95%. Isolated progenitors were cultured in RPMI 1640 (Gibco) supplemented with 10% heat-inactivated foetal calf serum (Gibco), 5×10^5 M 2-mercaptoethanol (Sigma), 100 U penicillin per ml and 100 μ g streptomycin per ml. Cultures were supplemented with recombinant GM-CSF (200 U/ml, specific activity 2×10^6 U/mg; Genzyme, Cambridge, MA, USA), and recombinant TNF α (50 U/ml, specific activity = 2×10^7 U/mg; Genzyme).

PGP9.5 immunostaining

All staining procedures were carried out on ice. Phosphate buffer saline containing 1% bovine serum albumin and 0.1% sodium azide was used for antibody dilution and washing. At day 1, 2 and 3, the suspensions enriched in LCs were pelleted by centrifugation (400 g for 10 min), incubated with normal goat serum, washed and incubated with anti-PGP9.5 antibody (Ultraclone) and/or phycoerythrin-conjugated anti-CD1a antibody (Dako, Trappes, France) for 1 h at 4°C. After 2 washes, cells were incubated for 30 min with FITC goat anti-rabbit IgG (1/50) (Zymed, San Francisco, USA). As a negative control, normal rabbit Ig fraction was used in place of anti-PGP9.5. Cells were subsequently washed and fixed in 1% paraformaldehyde. Each day, the same immunostainings were performed on cells issued from CD34+ cells in culture. Each experiment was repeated 10 times for epidermal LC and CD34+ derived cells.

Flow cytometry

Cells were analysed using a FACSTAR (Becton-Dickinson). Data acquisition was triggered by cell size (forward vs. 90° light scatter) in order to eliminate cell debris. For each assay, the value of the fluorescence intensity corresponded to the mean fluorescence intensity. The percentage of positive cells was determined by comparison with the negative control. The statistics were calculated using over 10^4 cells.

RESULTS

PGP9.5, ubiquitin carboxyl terminal hydrolase, was expressed by LC suspensions. A double staining revealed that only CD1a+ cells, i.e. LC, but not keratinocytes were able to express PGP9.5. At day 1, the expression of PGP9.5 was shown on $9.2 \pm 4.3\%$ of CD1a+ cells and increased at day 2 ($15.4 \pm 4.4\%$) and day 3 ($29.5 \pm 7.9\%$), as shown in Fig. 1.

Cultures of CD34+ cells were carried out for 14 days, according to the method described previously (3). Within 4–5 days, part of the cells formed clusters with peripheral cells displaying typical dendritic cell morphology. The number and size of aggregates increased until day 12, while a few adherent cells with long dendritic projections also progressively appeared. At days 12–14, a small percentage of dendritic cells/LC was generated, characterized by their expression of CD1a antigen. The proportion of cells expressing CD1a

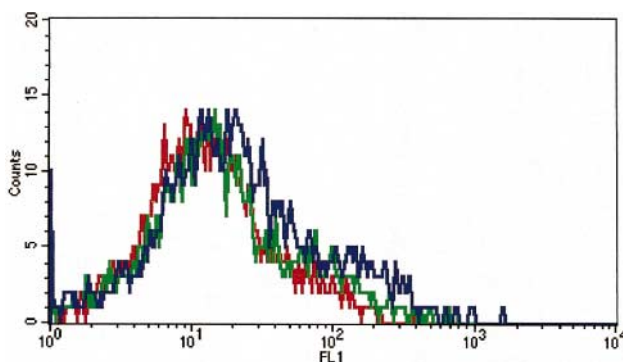


Fig. 1. Expression of PGP9.5 on cultured human Langerhans' cells at day 1 (red), day 2 (green) and day 3 (blue). In this experiment, percentages of PGP9.5+ cells, calculated after subtraction of negative controls (rabbit Ig fraction), were 9.1 at day 1, 15.41 at day 2 and 29.52 at day 3.

increased from 0% at day 0 to $20.6 \pm 5.4\%$ at day 6 and $75.0 \pm 9.4\%$ at day 12 (mean \pm SD, $n=10$). On any of the 14 days of the culture, CD1a+ dendritic cells, i.e. LC, never express PGP9.5.

DISCUSSION

Thus, LC are able to express PGP9.5 if they are in suspension, whereas their precursors do not express this protein at any stage of their differentiation. The expression of PGP9.5 was increased on cultured LC, i.e. mature dendritic cells.

In the normal epidermis, LC are connected to nerve fibres (4, 5). They do not express PGP9.5, but are closely associated with PGP9.5+ nerve fibres through their dendrites and cell body. If they are denervated, epidermal LC are able to synthesize PGP9.5, whereas they remained non-immunoreactive for nerve growth factor-receptor (16). When new axons grew into the epidermis after denervation, both PGP9.5+ axons and LC could be observed (16). Preparation of LC suspensions needs dismantling of the epidermis and thus the loss of contacts between LC and nerve fibres. Hence, suspensions of LC are equivalent to denervated LC. We do not know why PGP9.5 expression may be observed only on a fraction of CD1a+ cells. No LC in the epidermis are innervated (4, 5). We suggest that only LC which were innervated could express PGP9.5 after denervation.

PGP9.5 is a hydrolase, but its function in nerves remains unknown. Denervation might induce LC changes, whereas interactions between LC and nerve fibres are necessary for homeostasis. After denervation, LC might exert a trophic role on neurons and might be a target of growing axons in the epidermis (16). However, PGP9.5 appears to be not specific to nerve cells.

The enhancement of PGP9.5 expression on LC after 1 or 2 days in culture suggests that PGP9.5 may be a marker for maturation. Indeed, whereas fresh LC are immature, dendritic cells, LC maintained in culture are mature dendritic cells and resemble interdigitating cells of lymph nodes (17).

DC issued from CD34+ cells never expressed PGP9.5, even after 12–14 days of culture, when part of cells coming from CD34+ precursors in culture are equivalent to LC, as assessed by the co-expression of CD1a, CD83 and Birbeck granules (3, 18, 19). We suggest that the ability to express PGP9.5 needs 2 events. The first is the epidermal maturation of dendritic cells through cytokines, growth factors or neuromediators produced by epidermal nerve cells, keratinocytes or Merkel cells. The second is the lack of contacts with PGP9.5 nerve fibres. Under these conditions, LC are able to produce PGP9.5. Hence, PGP9.5 synthesis by LC is probably induced by an epidermal factor released in the absence of epidermal innervation.

When Paul Langerhans described the future LC in 1868 (20), he believed them to be nerve cells. The expression of PGP9.5 by LC does not confirm this hypothesis, but confirms that LC possess neuronal properties or potencies.

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