Immunophenotypic analysis of normal human dendritic cells isolated from epidermis and dermis

Report

Immunophenotypic analysis of normal human dendritic cells isolated from epidermis and dermis

Francesa Prignano, MD, PhD, Gianni Gerlini, MD, Nicola Pimpinelli, MD, PhD, Benvenuto Giannotti, MD, and Paolo Romagnoli, MD

From the Department of Human Anatomy and Histology and the Dermatology Clinic, University of Florence, Florence, Italy

Correspondence
Francesca Prignano, MD, PhD
Dipartimento di Anatomia ed Istologia
Sezione di Istologia "E. Allara"
Viale Pieraccini, 6
50139 Firrenze
Italy

Abstract

Background The skin immune system comprises two types of dendritic cells, i.e. CD1a-positive Langerhans cells in the epidermis and CD36-positive dendritic macrophages in the dermis. Dendritic cells can migrate from skin explants into a culture medium.

Methods We have examined the morphology and immunophenotype of the dendritic cells migrating from epidermal and dermal sheets *in vitro*. The epidermis and dermis of keratomes of normal human skin were separated with dispase and cultured for 72 h. At this time, the non-adherent cells in the medium were removed, enriched on a metrizamide or Lymphoprep gradient, counted, prepared by cytospin, and labeled for CD1a, CD36, and HLADr.

Results Cells migrating from the epidermis and dermis show many thin projections or a few veils from the cell surface. Approximately four times more cells migrate from epidermal than dermal sheets from the same keratome.

Conclusions Using methods to separate the epidermis from the dermis, both CD1a-positive Langerhans cells and CD36-positive dendritic macrophages can be obtained from both tissues, although in different numbers.

The skin immune system (SIS) is made up of numerous cell types which share an origin from bone-marrow precursor cells and can differentiate and mature under definite conditions.[1] The SIS includes dendritic cells, scavenger macrophages, and mast cells. The epidermal dendritic cells, i.e. Langerhans cells, bear major histocompatibility complex I (MHC-I) and MHC-II antigens and are the only cells in the epidermis to express the CD1a antigen. They carry antigens to draining lymph nodes and present them to CD4-positive lymphocytes within the framework of MHC-II molecules. Another group of dendritic cells in the dermis, mainly located around blood vessels, expresses the CD36 antigen and rarely, or not at all, CD1a, in addition to MHC-I and MHC-II antigens;[2] some authors have proposed that these cells can interact with CD8-positive lymphocytes.[3]

Several procedures have been used to isolate Langerhans cells from the skin with varying results.[4–6] One of these procedures involves the culture of epidermal explants and the collection of the cells migrating into the medium. It is well established that dendritic cells can migrate from epidermal sheets and can be collected after a definite period of time (which should not exceed 72 h, otherwise they degenerate[7]). High numbers of dendritic cells also migrate from dermal explants; a relatively large fraction of these cells has been reported to express the CD1a antigen and a very few the CD36 antigen,[7] which seems to be at variance with the results of immunocytochemical analyses on skin sections.[8] To address further the issues of the morphology and immunophenotype of cells migrating from explants of the epidermis and dermis, we have analyzed the cells migrating from epidermal and dermal sheets obtained from keratomes of normal human skin.

Materials and methods

Reagents and antibodies

Dispase, grade II, was purchased from Boehringer-Mannheim, Mannheim, Germany; RPMI 1640, amphotericin-B,and fetal calf serum (FCS) were obtained from Seromed, Berlin, Germany; Lymphoprep- was received from Nycomed Pharma, Oslo, Norway; penicillin–streptomycin and fluorescent isothiocyanate (FITC)-labeled goat anti-mouse secondary antibodies were purchased from Sigma, Milan, Italy; primary antibodies against CD1a, HLADr, CD36, and CD3 were obtained from Ortho Diagnostic Systems, Raritan, NJ.

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Cell cultures

Keratomes of normal human skin were obtained at plastic surgery, and part (approximately 2 cm^2 each) was used for this study after informed consent, following Italian law and the ethical guidelines of the Italian National Medical Council. The keratomes were incubated in a solution of dispase, 1.2 U/mL, in RPMI 1640 with 10 U/mL penicillin, 10 mg/mL streptomycin, and 50 μg/mL amphotericin-B, at 37 °C. The epidermis was easily separated from the dermis. The sheets were washed many times in phosphate-buffered saline (PBS) and cut into small pieces. The sheets were then cultivated separately in Petri dishes (10 cm in diameter) in RMI 1640 supplemented with 10% FCS and the same concentrations of the antibiotics. After 72 h, the sheets were removed and the medium was collected and centrifuged (160*g* for 10 min at 20°C) in a Hettich Universal 30 RF centrifuge. The cells were resuspended in 3 mL of fresh medium. The cell number and viability were assessed with trypan blue staining in a hemocytometer. A further enrichment of dendritic cells was obtained by separation with either hypertonic 14.5% metrizamide or 10% Lymphoprep; the cell suspension was laid over metrizamide or Lymphoprep and spun at 160*g* for 10 min at 24 °C. The low-density cells at the interphase were collected and, after two more washes in RMI 1640, were supplemented with 10% FCS and NaCl (40 and 25 mM respectively); cytospins were prepared, air dried, fixed in cold acetone for 5 min and stored at –20 °C until labeling. The cells contained in all the cytospins of each tissue culture of two experiments were counted by phase contrast microscopy, at a magnification of 40×, before labeling.

Immunohistochemistry

After rehydration, the cytospins were rinsed three times with 138 mmol/L NaCl, 2 mmol/L KCl, and 10 mmol/L PBS, and incubated with the mouse monoclonal antibodies (see above for a list of the antibodies) for 1.5 h at 37°C, followed by fluoresceinated secondary antibodies for 1 h at 37°C. The slides were mounted with Gel/mount (Biomed, Foster City, USA) and photographed in a Zeiss Axioskop microscope (Oberkochen, Germany) equipped for epifluorescence.

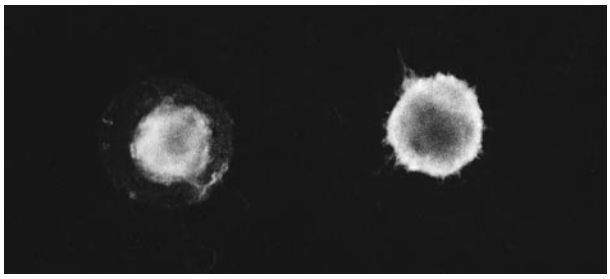

Figure 1 Two cells which had migrated from the dermis after 72 h culture; they appear veiled (on the left) or with many short dendrites (on the right). Immunofluorescence for HLADr, × 1000

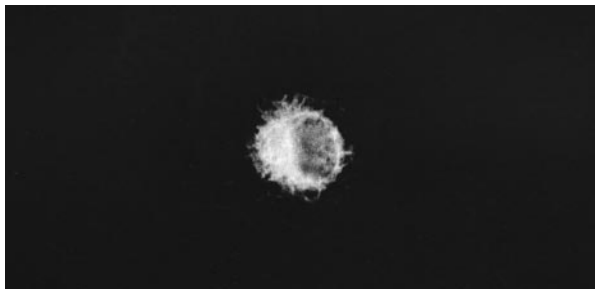

Figure 2 A dendritic cell which had migrated from the epidermis and expressed the CD1a antigen on the cell surface and body, excluding the nucleus. Immunofluorescence for CD1a, × 1000

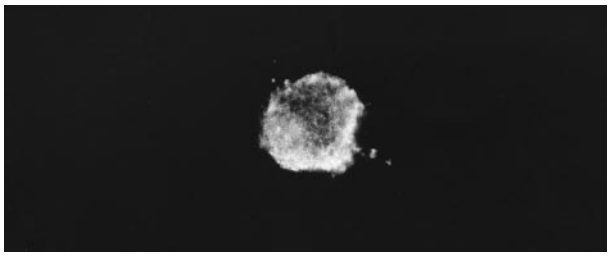

Figure 3 A dendritic cell which had migrated from the epidermis and expressed the HLADr antigen on the cell surface only. Immunofluorescence for HLADr, × 1000

Results

After 72 h of culture, cells had migrated from the epidermis and the dermis into the culture medium; they showed many short dendrites or, more rarely, appeared veiled (Fig. 1). More cells, usually better preserved in morphology, were obtained when using Lymphoprep, rather than metrizamide to enrich the dendritic cells. Because of this, definitive counts of cells migrating from epidermal and dermal sheets were obtained from two samples enriched with Lymphoprep. In these samples, the numbers of cells collected from the epidermis were much larger (about three times) than those collected from the dermis. The former numbered 2.1×10^4 in one experiment and 5.7×10^4 in another, and the latter numbered 6.5×10^3 and 1.8×10^4.

Many cells migrating from the epidermal expressed the CD1a antigen (39.36%; standard error, ± 8.68%; Fig. 2); less numerous were the HLADr-positive (26.72 ± 13.45%; Fig. 3) and the CD36-positive (11.35 ± 5.33%) cells. The majority of the cells migrating from the dermis expressed HLADr antigen (52.28 ± 19.78%; Fig. 1); many expressed the CD36 antigen (36.37 ± 13.64%), and a few the CD1a antigen (17.26 ± 1.08%). When detected, the labeling was always intense.

Discussion

The results of this study show that many dendritic cells migrate from epidermal and dermal sheets of human skin. Moreover, both CD1a-positive cells and CD36-positive cells were identified among the cells migrating from both sheets.

In this study, we regularly obtained more cells from the epidermis than from the dermis; this is at variance with previous investigations,[7] and may be attributed to differences in the dermal thickness removed from the keratomes.

Although we cannot exclude cross-contamination between the epidermis and dermis during the preparation of the sheets, this does not seem a probable hypothesis to explain the fact that both CD1a-positive and CD36-positive cells were found among the cells migrating from the epidermal and dermal sheets. We have checked the quality of our sheets by re-embedding, cross-sectioning, and light microscopy examination; although, in some instances, part of the basal epidermal layer had detached together with the dermis, dermal remnants were not found attached to the epidermis (data not shown). Therefore, some CD1a-positive ells migrating from epidermal explants may be epidermal Langerhans cells retained in the dermis, but CD36-positive cells migrating from the epidermis must have originally been in this location. Since the number of CD36-positive cells in the normal epidermis is very low compared with the number of CD1a-positive Langerhans cells,[9] the former are probably more prone to migrate from the cultured epidermis than the latter.

A wealth of data supports the existence of a compartmentalization of dendritic cells in the human skin, CD1a-positive Langerhans cells being predominantly in the epidermis and CD36-positive dendritic macrophages in the dermis;[10] however, both types of cell seem to exist in both compartments. Their relative number *in situ* is not likely to be reflected in the relative number of cells collected from the culture medium, because it is probable that many Langerhans cells in the dermis are already migrating (to or from the epidermis), and therefore are more prone to continue migrating from the dermal sheets than resident dermal macrophages. The opposite is true for the epidermis. Because the separation of the epidermis from the dermis was achieved in this study by widely used methods, which are routinely applied in the isolation of dendritic cells by tissue dissociation,[7] we conclude that it is doubtful that these methods can lead to a pure population of Langerhans or CD36-positive dendritic macrophages. Even if *in vitro* data cannot be compared directly with *in vivo* findings, however, the method illustrated here has the potential to allow for comparison of the *in vitro* behaviour of dendritic cells among different clinico-pathologic conditions.

The total number of HLADr-positive cells from the epidermis is definitely less than the sum of CD1a-positive and CD36-positive cells; this implies that at least some of these two cell types do not express the HLADr antigen. Discrepancies between the number of HLADr-positive and CD1a-positive cells in the epidermis have been described previously,[11] and may well explain the results of this study. This problem should not arise in the dermis, where the number of HLADr-positive cells is approximately similar to the sum of CD1a-positive and CD36-positive cells.

Finally, Lymphoprep proved to be superior to metrizamide for dendritic cell purification from the culture medium. In particular, the former gave more consistent results than the latter in terms of cell number and morphology. This finding seems in line with the fact that Fycoll-Hypaque and Lymphoprep are increasingly being used by different laboratories for the separation of dendritic cells.

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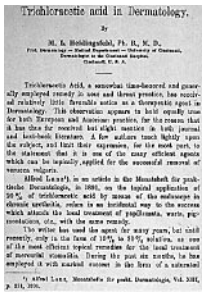
Trichloracetic Acid in Dermatology of 1911


Trichloracetic acid in Dermatology.

By

M. L. Heidingsfeld, Ph. B., M. D.

Trichloracetic Acid, a somewhat time-honored and generally employed remedy in nose and throat practice, has received relatively little favorable notice as a therapeutic agent in Dermatology. This observation appears to hold equally true for both European and American practice, for the reason that it has thus far received but slight mention in both journal and text-book literature. A few authors touch lightly upon the subject, and limit their expression, for the most part, to the statement that it is one of the many efficient agents which can be topically applied for the successful removal of verucca vulgaris.

Alfred Lanz[1] in an article in the Monatsheft für praktische Dermatologie, in 1891, on the topical application of 20% of trichloracetic acid by means of the endoscope in chronic urethritis, refers in an incidental way to the success which attends the local treatment of papillomata, warts, pigmentations, etc., with the same remedy.

The writer has used the agent for many years, but until recently, only in the form of 10% to 20% solution, as one of the most efficient topical remedies for the local treatment of mercurial stomatitis. *From Heidingsfeld ML. Trichloracetic acid in dermatology. Arch Dermatol Syphil 1911; 110: 245–246.*

Trichloracetic acid has received relatively little favorable notice as a therapeutic agent in European and American Dermatologic journal and textbook literature at the beginning of this century, although employed generally in nose and throat practice. Only few articles praised its topical potency (up to 20% solution) in the removal of warts, papillomata, pigmentations and in the endoscopic treatment of chronic urethritis. Heidingsfeld used it in the form of saturated solution in a large number of dermatoses. Trichloracetic acid precipitates albumin most efficiently by direct contact and can be most advantageously employed for the destruction and removal of exuberant and pathogenic cell tissue. Used only for small warts and moles in the beginning, its efficacy and excellent cosmetic effect encouraged the writer to extend its use to some refractory infections of the skin, such as lupus erythematosus, lupus vulgaris, Paget's disease of the nipple, and many others with apparently good immediate results.

Comment

Since then many different sources have been applied in the treatment of the skin diseases mentioned above and some of them have been as well rejected again. Today we should be aware of the histopathology of pigmented lesions before treatment and of the recurrence rates after insufficient treatment of malignant diseases and therefore trichloracetic acid application is limited. We still use trichloracetic acid for the removal of superficial skin lesions, especially in the treatment of photodamaged skin.

Remark

The Austrian "Archiv für Dermatologie und Syphilis" was originally published in German but occasionally it featured articles by foreign contributors in their original language as a tribute to international dermatological literature.

Daisy Kopera, MD, Graz, Austria
Karl Holubar, MD, Vienna, Austria

