IMMUNOHISTOCHEMICAL IDENTIFICATION OF LANGEHANS CELLS IN NORMAL SKIN AND INVOLVED SKIN WITH PITYRIASIS LICHENOID CHRONICA AND PSORIASIS

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ABSTRACT

Ten skin biopsies from patients with psoriasis and pityriasis lichenoid chronica, five form each case, were studied in the present work. Control skin was obtained at surgery. All specimens were prepared for paraffin sections for routine histological examination, and immunoperoxidase staining to investigate the pattern of distribution of Langerhans cells (LCs) in normal and involved skin with psoriasis and pityriasis lichenoid chronica.

Normal human skin in immunoperoxidase stained sections, revealed regular suprabasal distribution of Langerhans cells in the epidermis.

In haematoxylin and eosin stained sections of psoriatic plaques, there was hyperkeratinization, hypopigmentation and dermal mononuclear cell infiltration. Immunoperoxidase stained sections of psoriatic cases showed uneven epidermal distribution of Langerhans cells. Some epidermal areas were devoid of LCs, others showed few scattered cells, while another areas showed large number of clumped interdigitated LCs. In these cases immunoperoxidase positive cells were also detected in the upper part of the dermis and in dermal papillae.

In the present study, sections from cases with pityriasis lichenoid chronica showed hypopigmentation together with dermal mononuclear cell infiltration. Sections of these cases showed also large number of immunoperoxidase positive LCs in the epidermis and among the cellular infla-
IMMUNOHISTOCHEMICAL IDENTIFICATION etc...

trate in the upper dermis.

Much further work remains to be carried out to characterize the LCs structure and functions. What is certain is that a wealth of data about LCs must now be looked at from a new viewpoint and it will hopefully result in understanding and significant insights into a variety of lesions conditions involving the skin, as for example, psoriasis and pityriasis lichenoid chronic.

INTRODUCTION

Langerhans cells (LCs) were initially described by Langerhans,[1] using gold chloride staining method. He revealed a population of non pigmen-
tary, dendritic, suprabasal epidermal cells that are now referred to as Langerhans cells. Since that time there has been much speculation concerning the origin, associations and functions of these clear cells, situated in the higher levels of the epidermis [1].

Attempts were made to fit the dendritic (LC) into one of two already established systems of dendritic cells. One group of authors favored Langerhans suggestion that the dendritic "Clear cells" in the epidermis are neural in nature. Where as another group favored the view that these cells are derived from "effete" melanocytes [2].

Although initially considered to be related to melanocytes [3], present evidence favors a mesodermal origin for LC [4,5]. It is now accepted that these dendritic cells form a relatively constant population admixed with epithelial cells in stratified epithelium of the epidermis, esophagus and cervix [6,7]. In such sites, LCs appear to be capable of maintaining their position, while the surrounding cells keratinize and are sloughed off. It has been suggested that they may be a form of epidermal scavenger [8].

Unfortunately, the keratinocytes with which LCs form a close association are considerably more efficient at endocytosis of injected tracers. Thus, if the Langerhans cell is a macrophage, it is of the poorly phagocytic variety [9].

Other suggestions concerning LC function, include control of keratinization [10] and control of keratinocyte proliferation [11]. However, these theories either lack experimental corporation or fail to account for all the known facts concerning the LC type.

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It was prunieras [12] who first made the suggestion that antigens applied to the integument might interact with Langerhans cells. This speculation has recently been elegantly substantiated by Silberberg[13]. Furthermore, Silberberg et al[14] and Hunter et al[15] suggested an immunological function for LC in contact allergic reactions, where close relationship with T-lymphocytes is evident and the Langerhans cells might process antigens like the macrophages. Shelley & Juhlin[16], reported that Langerhans cells form a reticuloepithelial trap for external contact antigens.

Although considerable attention has been paid to the ultrastructural characteristics of epidermal Langerhans cells, little of the literature has identified the role of LCs in dermatitis [17].

Study of the mechanism underlying the interaction between epidermal LCs and cutaneous keratinocytes may provide a clue not only for understanding the pathogenesis of skin lesions like psoriasis and pityriasis lichenoid chronicum (PLC) but also for the elucidation of selected aspects of immunological functions of Langerhans cells[18].

Indeed, investigators, using a variety of techniques, have shown that, there was abnormality concerning LCS distribution and average percentage among other keratinocytes in involved skin with psoriasis and PLC[19,20].

To define further the specific lesions and to characterize Langerhans cell participation in psoriasis and PLC, the current study examined the cell population in well-developed lesions of both types of these skin diseases as well as in normal human skin by immunohistochemical means. A monoclonal antibody against human CD1a antigen was used in the present study.

CD1a antigen is expressed on epidermal dendritic cells and cortical thymocytes. The monoclonal antibody NC1-CD1a 220 stains the epidermal Langerhans cells, interdigitating cells of the dermis and interdigitating cells of stratified squamous epithelium of the tonsils [21].

MATERIAL AND METHODS
The current study involved 10 patients from the department of dermatology (Mansoura University hospit-
tals). They were chronic patients having repeated attacks of the skin eruptions and history of failed medical treatment. Skin biopsies for histopathological differential diagnosis were taken from them, after giving informed consent. For normal skin, sample was obtained at surgery.

Punch biopsy specimens 3-4 mm in diameter, were obtained from the cutaneous lesions. They were immediately fixed in 10% neutral buffered formalin, prepared for paraffin sections which were used for Hx & E staining.

The monoclonal antibody used in this work was NCL-CD_{1a}-220 which specifically detect Langerhans cells. It is produced by (Novocastra Laboratories Ltd., Newcastle, UK.)

Six μm paraffin sections from all specimens were stained by the preformed avidin-biotin-peroxidase complex method detailed elsewhere [22,23]. No specific peroxidase activity is first blocked by incubation of the sections with 3% H_{2}O_{2} in methanol for 20 minutes, at room temperature. After that section were blocked with normal horse serum diluted in phosphate-buffer salin (PBS) for 30 minutes at 37°C. Then, sections were incubated with a 1 : 40 dilution of anti-CD_{1a} antibody for 60 minutes at room temperature.

This was followed by 30-minutes separate incubations with biotin-conjugated horse anti-mouse IgG diluted in PBS buffer at 37°C and the preformed avidin-biotin-peroxidase complex at 37°C. The reaction product was revealed by incubation in 0.05M Tris-HCL buffer (pH 7.6), 0.05% DAB (diaminobenzedine) and 0.01% H_{2}O_{2} for 5 minutes at room temperature, followed by quick rinse in distilled water. Finally, after nuclear staining with Meyer's Hx., the sections were dehydrated, cleared and mounted in eukit.

RESULTS

(1) control thin skin :

Haematoxylin and eosin stained sections showed melanin pigment in the different strate of the epidermis of control thin skin (Figs : 1,2). The dermis comprised of superficial papillary layer and a deep reticular layer containing skin appendiges (Fig. 1).

The immunoperoxidase staining with anti CD_{1a} antibody demonstrated the positively stained cell bodies and
dendritic processes of Langerhans cells in the epidermis. Epidermal dendritic LCs were found to be randomly distributed among keratinocytes above the epidermal basal cell layer (Figs. 3, 4, 5, 6). These epidermal dendritic cells were never seen in the stratum corneum (Figs. 5, 6).

Similar positively stained dendritic cells were observed within the hair follicles (Fig. 3).

(2) psoriatic skin:

Involved psoriatic skin in Hx & E stained sections showed epidermal hypopigmentation in the form of almost complete absence of melanin pigment together with hyper keratinization represented by marked increase in the thickness and number of epidermal and horny layers with formation of horny strips or scales (Figs. 7, 8).

A prominent mononuclear cell infiltration was detected in the papillary layer of the dermis and in the upper part of the reticular layer of the dermis (Fig. 7).

Immunoperoxidase stained sections from psoriatic caes demonstrated uneven distribution of Langerhans cells in the epidermis of involved psoriatic skin (Figs. 9, 10, 11). In localized areas of the epidermis Langerhans cells formed intensely stained network in the suprabasal Malpighian layers. Other epidermal areas were devoid of immunoperoxidase positive LCs or contained, haphazardly scattered LCs (Figs. 9, 11).

However, they were more numerous in number compared to control sections. Immunoperoxidase positive cells were also detected as dense clusters in the dermis. They were encountered, mostly along the elongated dermal papillae, in the upper dermis and among other cells constituting the hair follicles. (Figs. 9, 10).

(3) skin involved with pityriasis lichenoid chronica:

Histological examination of haematoxylin and eosin stained sections from skin involved with pityriasis lichenoid chronica showed hypopigmentation with marked decrease of melanin pigment in the basal layers of the epidermis compared to control sections (Fig. 12). Irregularity of the horny layers with formation of mica plaques was detected (Fig. 12). There was moderate
mononuclear cell infiltration of the papillary and upper part of the reticular layers of the dermis (Figs. 13, 15, 16).

Throughout the epidermis of skin involved with pityriasis lichenoid chronica, there was large dendritic cells positively stained with anti-CD1a monoclonal antibody. Many of the immunoperoxidasepositive LCs exhibited strong positive staining of both their cell bodies and their dendritic processes (Figs. 13, 14, 15).

In addition, occasional bizarre shaped cells were noticed (Fig. 16). In the dermal infiltrates, similar less branched cells with fewer dendritic processes were also detected (Figs. 15, 16).

Fig (1) : A photomicrograph of a normal human skin. It is formed of two components. The dermis with its papillary layer (P) and reticular layer (R) containing hair follicles and the epidermis with its cellular and horney layers. (Hx. & E.; X100)

Fig (2) : A photomicrograph of normal human thin, skin. Showing the layers of epidermis and the normal distribution of melanin pigment. (Hx. & E.; X 250)
Fig (3) : A photomicrograph of control human skin. It reveals the immunoperoxidase positive Langerhans cells (lc) regularly distributed in the suprabasal layers of the epidermis. Few dendritic cells bind the antibody are seen within the hair follicles (arrow). (Anti CD1a antibody reaction; X 40)

Fig (4) : A photomicrograph of control human skin. Note that the dendritic Langerhans cells (arrow) are the only epidermal cells which bind specifically the anti CD1a anti body (arrow). Other epidermal cells and the dermal tissue show negative immunoperoxidase reaction. (Anti CD1a antibody reaction ; X 100)

Fig (5) : A higher magnification of the epidermis in ( fig. 4 ). It demonstrates the regular distribution of immunoperoxidase positive Langerhans cells (arrow) among the negatively stained keratinocytes of the human epidermis. (Anti CD1a antibody reaction; X 250)

Fig (6) : A photomicrograph of a control human skin, revealing immunoperoxidase positive Langerhans cells. Note the exension of the dendritic processes of these cells inbetween the keratinocytes. (Anti CD1a antibody reaction ; X 400)
Fig (7): A photomicrograph of human skin involved with psoriasis. It shows hypopigmentation, hyperkeratinization with marked increase in the thickness of the epidermis, formation of horny scales (hs) and infiltration of the dermis with mononuclear cells (m). (Hx. & E.; X 100)

Fig (9): A photomicrograph of a section of human skin involved with psoriasis. It shows uneven distribution of Langerhans cells throughout the epidermis. Note the strong positive immunoperoxidase reaction in the mononuclear cell infiltrate of the papillary layer of the dermis. Note: The thickened part of the horny layer forming psoriatic horny scales (hs) (Anti CD1a antibody reaction; X 100)

Fig (8): A photomicrograph of involved skin with psoriasis. It reveals the marked increase of number of epidermal layers if compared with the control section. (H x. & E.; X 250)

Fig (10): A photomicrograph of a section of human skin involved with psoriasis. It shows heavy mononuclear cell infiltration of the dermis (m). Immunoperoxidase positive cells are frequently seen in the hair follicles and among the mononuclear cell infiltrate (arrow). (Anti CD1a antibody reaction; X 100)
Fig (11) : A photomicrograph of section of human skin involved with psoriasis few of the dendritic Langerhans cells are separately distributed while many of them are clumped together(c). (Anti CD1a antibody reaction; X 400)

Fig (13) : A photomicrograph of a section of human skin involved with pityriasis lichenoid chronica. It demonstrates regular distribution of many dendritic langerhans cells within the epidermis(\textsuperscript{1}). Positive immunoperoxidase reaction is also detected in some cells within the hair follicle (arrow) and among the dermal infiltrate (arrow). (Anti CD1a antibody reaction; X 100)

Fig (12) : A photomicrograph of a section of human skin involved with pityriasis lichenoid chronica. It shows hypopigmentation with almost complete absence of melanin pigment and irregular formation of the horny layers mica plaque (mp). (Hx. & E.; X 400)

Fig (14) : A photomicrograph of a section of human skin involved with pityriasis lichenoid chronica. Langerhans cells are seen with their dendritic processes (arrow) extending among the epidermal cells. Similar positively stained cells are seen in the upper dermis. (Anti CD1a antibody reaction; X 250)

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DISCUSSION

The function and ontogeny of Langerhans cells have generated considerable interest over the last two decades. Hence, the origin and function of LCs have been extensively studied. They appear to be derived from bone marrow precursors which migrate to the epidermis and localize in its suprabasal portion[24]. This finding was subsequently confirmed using ultrastructural studies, disproving previous suggestions that the LC represented an effete melanocyte[3,4]. In addition, investigations done by Takamaki and Katz[25], have demonstrated that murine epidermal LCs are derived from precursor cells within the bone marrow and represent a renewable population within the skin.

Apparently, identical cells occur in
extra-epidermal situations including lymph nodes and the thymus[26,27]. Since Wolff's[5] Comprehensive review of the LC, attention has been drawn to the possibility of an immunological role for the cell in contact allergy.

Langerhans cells selectively take up the exogenous antigens causing allergic contact dermatitis and present these antigens to T cells[16]. They differ from ordinal monocytes and macrophages in the presence of distinctive cytoplasmic organelles, rod or racket shaped Langerhans-cell "birbeck" granules, and by being only moderately phagocytic[17].

Epidermal Langerhans cells are now considered to perform an important role in immunological reactions within the skin[28]. In vitro studies have revealed that LCs are able to present antigens to sensitized lymphocytes and to act as stimulator cells in mixed lymphocyte cultures[29,30]. They are considered essential for allergic contact sensitization[31] and may be targets in allogenic skin graft rejection [6]. Many authors have implicated the LC in the pathogenesis of cutaneous T cell lymphomas on the basis of morphological studies, but evidence for their functional role in these lymphomas is lacking [32].

The LC, which occurs regularly within mammalian keratinizing epidermis is morphologically, immunologically, and functionally distinct from both surrounding keratinocytes and melanocytes. Its dendritic morphology and even its distribution are particularly well illustrated by ATPase staining of EDTA-separated epidermal sheets[33].

Langerhans cells differ from normal melanocytes and Keratinocytes in that, they possess surface antigens and receptors that are known to be involved in immune reactions[34]. Recently it has been found that NCL CD1a 220, one of a number of monoclonal antibodies, selectively binds to human epidermal Langerhans cells. Of greater interest, is the absence of binding of this antibody to circulating blood leukocytes, including T-lymphocytes. The advantage of this Langerhans cell probe over previous stains and probes is that infiltration of the dermis or epidermis by leukocytes would not interfere with the LC specificity. Therefore, the use of NCL CD1a 220 antibody would not be limited to normal skin but could
be used to ascertain the presence of LCs within altered skin including inflammatory diseases[35]. The Latter authors have found detection of CD\textsubscript{1a} antigens on Langerhans cells and indeterminate cells in human thin skin a superior method than detection of Ia (HLADR) antigen expression.

Tagami and Alba[21] presented the data favoring the hypothesis that CD\textsubscript{1a} antigens detected on the keratinocytes surface are actively synthesized by LC cells. They also suggested that the previously mentioned immunological response accompanied by exocytosis of activated T-lymphocytes is a sign of some active role played by those keratinocytes in cellular immunity.

Evidence favoring the concept that Langerhans cells play a significant role in certain immunological reactions was put forward by Luger et al[36] and Volk Plater at al[37] who reported close apposition (peripolesis) of mononuclear lymphocyte-like cells to LCs within 3-5 hours of topical application of mercury bichloride, followed by cell to cell interaction and subsequent damage.

Quantitative studies of Langerhans cells in a variety of diseases have abounded recently with the authors attempting to correlate an increase in or paucity of LCs with the pathogenesis of various cutaneous diseases[17]. It therefore, seemed worth while to find out the way in which Langerhans cells are involved in the pathogenesis of various conditions of skin diseases. Hence, the present work investigated the distribution of LCs in normal skin and involved skin from pityriasis lichenoid chronic and psoriatic patients.

In normal human skin immunoperoxidase positive dendritic LCs were seen only in the suprabasal malpighian layers of the epidermis and within the hair follicles. This finding is in agreement with the work done by Brown et al[38]. They reported that LCs constitute a fairly fixed population in the epidermis and pilosebaceous system. They appear to be absent in the dermis under normal conditions. Furthermore, in accordance with the present work, Jimbo and his coworkers[39], could not find one single LC in the dermis in the large number of normal human sections they examined. On the contrary those authors clearly showed that LCs are present in the dermal infiltrates of mycosis
fungicides, and that these cells appear to be able to cross over the epidermodermal junction. The latter cells may represent dermal LCs or their precursors.

In this current work, immunoperoxidase stained sections from a psoriatic plaque revealed that, the distribution of LCs was uneven. Some segments of the epidermis were devoid of dendritic cells, while others showed randomly distributed LCs. Another segments of the involved psoriatic epidermis showed clusters of interdigitated LCs. Moreover, clusters of immunoperoxidase positive cells were revealed in the dermal papillae and upper dermis especially surrounding the mononuclear cell infiltrates.

In immunoperoxidase stained sections taken from the hypopigmented plaques of pityriasis lichenoid chronic, no cytoplasmic staining of keratinocytes for CD\textsubscript{1a} antigens was detected as in normal and psoriatic skin. However, large number of positively stained LCs were detected in the epidermis. They exhibited morphological alterations. Most important among these changes were reduced number of surface dendrites and the occasional presence of bizarre shaped. Cells Also, as in psoriatic cases, there was immunoperoxidase positive cells in the upper part of the dermis of skin from cases of PLC encircling the mononuclear cell infiltrates. Bhan et al[35], reported that these cells may represent the so-called "indeterminate" or Langerhans precursor cells.

We concluded that these morphologic alterations of LCs together with the dermal mononuclear cell infiltration in PLC and psoriatic lesions represent specific changes rather than a simple inflammatory response. As a consequence we can confirm in agreement with Sontheimer & Bergstresser[40] and Hafter et al[41] that the above mentioned clinical lesions as well as the underlying histopathological alterations result from antibody or immune complex-mediated autoimmune injury.

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الاظهار الهستوكيميائي المناعي لخلايا لانجرهانز في الجلد الطبيعي والمصاب بالصدغية ومرضى النخالة الجزائر المزمن

د. سناء أحمد الشربيني

من قسم الهستولوجيا - كلية الطب - جامعة المنصورة

في هذه الدراسة تم فحص عينات من الجلد أخذت من خمسة مرضى مصابين بمرض الصدغية وخمسة عينات أخرى أخذت من مصابين بمرض النخالة الجزائر المزمن، بينما تم الحصول على عينات الجلد الضيقة أثناء العمليات الجراحية.

وقد تم إعداد وتحضير كل العينات للحصول على قطاعات شمعية استخدمت بعضها للدراسة الهستولوجية المعتادة، بالإضافة إلى ذلك أُستخدمت بعض القطاعات الشمعية لإجراء تفاعل البيبراكسيداز المناعي لتحري النمط الترويجي لخلايا لانجرهانز في الجلد الطبيعي والمصاب بالصدغية ومرضى النخالة الجزائر المزمن.

وقد ظهرت خلايا لانجرهانز موزعة بشكل منتظم في الخلايا فوق الأدمة لجبل الإنسان الطبيعي في القطاعات المصورة بتفاعل البيبراكسيداز المناعي في القطاعات المصورة بالهيماتوكسيلين والأيزوسين لجرير الصدغية لوحظ إضحال صفيي وزيادة في الطبقات الكيراتينية وتحلل أدمج الجلد بالخلايا وحيدة الأثر. وقد أظهر تفاعل البيبراكسيداز المناعي لحالات الصدغية توزيع غير منتظم لخلايا لانجرهانز ظهرت أجزاء من الطبقة فوق الأدمة خالية تماماً من خلايا لانجرهانز وأجزاء ثانوية ظهر بها عدد قليل من تلك الخلايا بينما أظهرت أجزاء أخرى على عدد كبير من خلايا لانجرهانز متجمعة ومتداخلة مع بعضها في الدراسة الحالية، أظهرت القطاعات المأخوذة من حالات مرضى النخالة الجزائر المزمن إضحال صفيي وتحلل الأدمة بالخلايا وحيدة الأثر. وقد أظهرت القطاعات هذه الحالات عددًا كبيرًا من خلايا لانجرهانز في الطبقة فوق الأدمة وأيضاً فيما بين الخلايا وحيدة الأثر في أعلى طبقة الأدمة.
ويبقى الكثير من البحث الواجب أداءه للوصول إلى مرا Boehlen ووظائف خلايا لانجرهايز والمؤكد في هذا الصدد أن تلقا من المعلومات المتعلقة بخلايا لانجرهايز والكمسة لديها يجب النظر إليها الآن من متوفر جديد على أمل الحصول على فهم ورؤية مفيدة للعديد من الحالات الجلدية، مثال ذلك سرطان الصدفي وال الجهاز الجزاء المزمين.