ORIGINAL ARTICLE

CD1a+ Langerhans Cells in the Peritumoral Epidermis of Basal Cell Carcinoma

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Abstract. *Background.* Basal cell carcinoma (BCC) is a common malignant tumor and its incidence has risen in recent decades. Research has shown the relationship between ultraviolet (UV) radiation, the skin immune system, and BCC. The role of Langerhans cells (LC) in the immune response to tumors has prompted research into LC density and morphology in response to UV radiation and BCC. However, the data are inconsistent due to differences in research methodology.

Objective. To study the density and morphology of LCs in the peritumoral epidermis of BCC using immunohistochemistry and image processing software and compare the results with those from the epidermis overlying the tumor.

Material and methods. Twelve samples from patients with BCC were prepared with a CD1a stain. Areas of epidermis overlying and adjacent to the tumor were defined using light microscopy and the Image J image processing software. The LCs in each area were counted and the cell densities were calculated and compared. Morphological features of LCs were also evaluated in each epidermal area.

Results. The results showed a lower density of LCs in the epidermis overlying the tumor than in the peritumoral epidermis ($P < 0.05$). There were also differences in the size, shape, and dendritic pattern of the LCs between the epidermal areas.

Conclusions. The lower density and fewer morphological changes of LCs in the epidermis overlying BCC may give rise to alterations in the immune response to BCC. Digital image analysis is a reliable method for the morphometric evaluation of LCs.

Key words: basal cell carcinoma, Langerhans cells, density.

CÉLULAS DE LANGERHANS CD1A+ EN LA EPIDERMIS PERITUMORAL DEL CARCINOMA BASOCELULAR

Abstract. *Introducción.* El carcinoma basocelular (CBC) es un tumor maligno frecuente y su incidencia ha aumentado en las últimas décadas. Investigaciones han demostrado la relación entre radiación ultravioleta (RUV), sistema inmune cutáneo y CBC. La función de las células de Langerhans (CL) en la respuesta inmuneantitumoral ha motivado la investigación de su densidad y morfología frente a la RUV y al CBC. Sin embargo, los resultados publicados presentan discordancias debido a diferencias en la metodología científica.

Objetivo. Estudiar la densidad y morfología de las CL en la epidermis peritumoral comparada con la epidermis supratumoral mediante inmunohistoquímica y un programa computacional de imágenes digitales.

Material y métodos. Doce casos de CBC fueron teñidos con CD1a. Se utilizó microscopia óptica y el programa computacional Image J para calcular las áreas epidérmicas sobre el tumor y adyacente a él. Se contabilizaron el número de CL de cada área y se determinaron y compararon las densidades celulares. Finalmente, se analizó la morfología de las CL en ambas áreas epidérmicas.

Resultados. Los resultados mostraron una menor densidad celular en la epidermis supratumoral respecto a la peritumoral ($p < 0,05$). Las CL entre ambas áreas presentaron diferencias en el tamaño, forma celular y patrón dendrítico.

Discusión. La menor densidad y alteraciones estructurales de las CL supratumorales podrían dar lugar a variaciones de la respuesta inmune en el CBC. El uso de tecnología de análisis de imágenes digitales sería un método fiable en el estudio morfométrico de las CL.

Palabras clave: carcinoma basocelular, células de Langerhans, densidad.

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Introduction

Basal cell carcinoma (BCC) is the most common malignant tumor affecting humans,[1] accounting for 70% to 80% of skin tumors in North America.[2,3] Epidemiology studies have shown a relationship between exposure to UV radiation and the onset of BCC.[4-6] UV radiation is the most important and widely studied cause of BCC, and it can produce a series of effects such as direct damage to cellular DNA, altered cutaneous immune surveillance, and cellular inflammation.

Langerhans cells (LC) are a key element in the functioning of the cutaneous immune system, given their ability to process different types of antigen, migrate to lymphoid tissue, and present antigens to T lymphocytes. The structural and functional alterations of LCs caused by UV radiation lead to abnormal behavior, affecting the different phases of the epidermal immune response.

The LCs in the epidermis of these tumors have been analyzed using different immunohistochemical markers such as the monoclonal antibody anti-T6, S100, langerin, and CD1a.[7-10] The latter has the advantage that, in addition to being a specific surface marker of LCs, it also plays a role in the antigen presentation to T lymphocytes.[11] The results of published studies on LCs in BCC are contradictory, but the discrepancies observed are due to the different methodological approaches followed.

Some studies show a reduction in the density of LCs in the epidermis overlying the tumor mass in comparison with the peritumoral epidermis or healthy skin.[7,10,12-14] Others have found no differences in the number of epidermal LCs between benign and malignant cutaneous lesions,[15,16] or they have reported increased density of LCs in the epidermis overlying BCC.[17,18] Finally, cutaneous immune system dysfunction in the context of UV radiation and BCC has also been inferred by studying the morphology of LCs, with reports of changes in cell size and dendritic pattern that reflect their inability to act as antigen presenting cells.[9,10,12,15]

The objectives of the present study were to examine the density and morphology of LCs in the epidermis overlying BCC and to compare the results with those for the peritumoral epidermis in the same patient.

Material and Methods

Case Reports

We requested BCC samples from the Histopathology Department of the Hospital Clínico of the Universidad de Chile in order to assess the feasibility of analyzing LCs using immunohistochemical staining with CD1a. Following to the protocol of the Histopathology Department, excised tumors are fixed in 10% buffered formalin and cut perpendicular to the major axis of the sample at 1-mm intervals, obtaining histologic slides that cover the whole sample.

We requested all reported cases of nodular BCC from the period 2000 to 2005.

The tumors were selected in accordance with a series of inclusion and exclusion criteria. The inclusion criteria were as follows:

1. 100% nodular tumors
2. The section selected contained the greatest area of tumor.
3. The sample had to have an intact epidermis both overlying the tumor mass and adjacent to it.
4. The selected tumors had to be completely excised.
5. Tumors had to have been on the face.
6. The tumors had to be from patients with similar occupations and who had lived in the same area all their lives.

The exclusion criteria were as follows:

1. Tumors with mixed histologic types
2. Patients with immunosuppression resulting from phototherapy, radiotherapy, organ transplant, or chronic corticosteroid therapy

We finally selected 12 cases for study —these were from 7 men and 5 women, with a mean (SD) age of 63 (11.5) years (range, 42-82 years).

Immunohistochemistry Technique

The samples were fixed in formalin for 24 hours before being embedded in paraffin. Three-millimeter thick sections of each tumor were taken and stained using the 3-step streptavidin-biotin immunohistochemistry technique. Antigens were recovered using a digital decloaking chamber (Biocare Medical, Walnut Creek, California, USA) using Reveal (Biocare Medical). Enzymes were then recovered using pepsin (Carezyme II, Biocare Medical) at room temperature.

The sections were incubated for 2 hours with monoclonal antibody CD1a, CLONE MTB1 (Novocastra monoclonal mouse), in Revival Series Van Gogh Yellow Diluent (Biocare Medical) at a dilution of 1:80. The secondary antibody used was a biotinylated antibody of 4plus Universal Immuneperoxidase Detection System (Biocare Medical) incubated for 20 minutes. The preparations were finally incubated in the 4 plus Universal Immuneperoxidase Detection System Streptavin-Enzyme Conjugates (Biocare Medical) for 20 minutes.
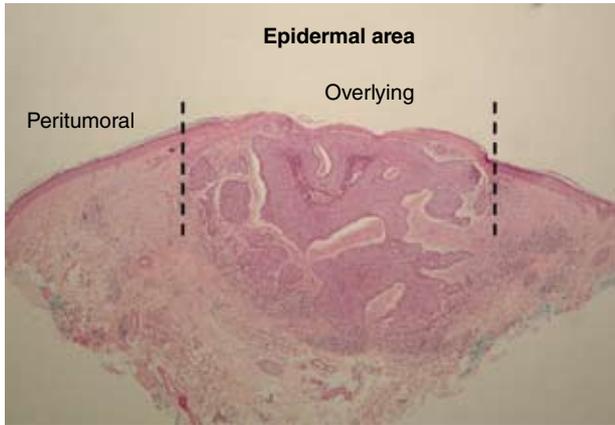
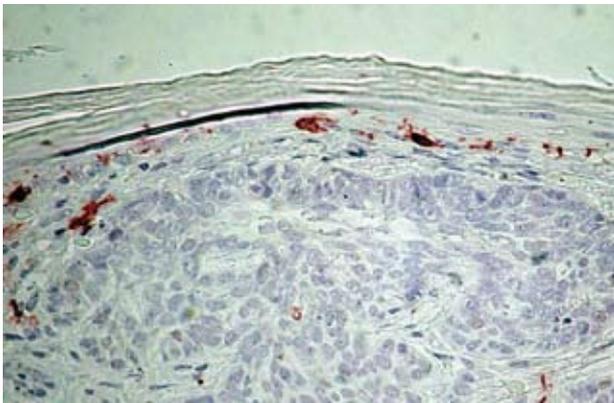**Figure 1.** Determination of Langerhans cells in the overlying and peritumoral epidermis (hematoxylin-eosin, ×40).

Figure 2. Langerhans CD1a+ cells in the overlying epidermis (CD1a, ×1000).

NOVARED was used as a chromogen, with an incubation time of 10 minutes.

Determination of the Density of the LCs

Determination of the Epidermal Area

Two epidermal areas were defined for study (Figure 1):

1. Overlying area: area of the epidermis overlying the tumor mass
2. Peritumoral area: area of the epidermis up to approximately 2500 μm from one border of the tumor mass

Each case was photographed using a digital camera connected to a light microscope (magnification, ×100).

The image processing software package Image J was used to determine the study areas by marking both complete epidermal surfaces and providing the measurement in pixels.

A relationship was established between pixels and μm² in order to express the epidermal area in μm². A Neubauer chamber was used, and a previously determined area was photographed (at the same magnification). This area was then analyzed using Image J, thus providing a relationship between pixels and μm².

Cell Quantification

At a higher magnification (×400), in each epidermal area, only those cells with a stained nucleus containing 2 or more dendrites were counted as LCs. This criterion was applied to all areas studied. Tumors were coded and the count was made blind by a single observer (FM).

Each count was repeated 5 times, and the average number of cells was established for each individual case.

Statistical Analysis

The t test for paired samples was used to compare the density of LCs in the 2 study areas in each case. Statistical significance was set at a P value less than .05.

Results

The LCs in the epidermis overlying the tumors had a morphologic appearance that was different to that of the peritumoral LCs (Figures 2 and 3). The cell body was large, rounded, or irregular, with less numerous, less elongated dendrites with a lower number of ramifications. LC density in each tumor was lower in the overlying epidermis than in the peritumoral epidermis (P<.001) (Table 1).

Intratumoral LCs were also observed (Figure 4).

Discussion

Through their participation in the cellular immune response, LCs play a central role in cutaneous immune surveillance. The epidermal density of LCs has been associated with epidermal immunologic reactions such as sensitization phenomena and immunosuppression.[18] The latter has also been associated with a greater risk of developing skin cancer.[19,20] One of the most studied factors in the pathogenesis of BCC is localized cutaneous immunosuppression induced by UV radiation; a number of publications have associated this phenomenon with quantitative, morphologic, and functional changes in LCs.[8,15,16]
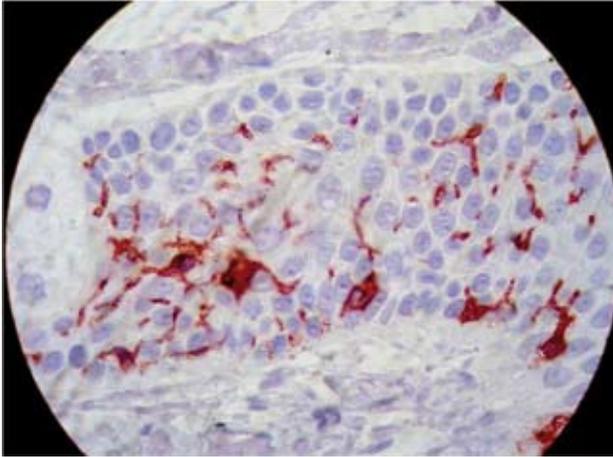
Figure 3. Langerhans CD1a+ cells in the peritumoral epidermis (CD1a, ×1000).

Table 1. Density of Langerhans Cells in the Overlying and Peritumoral Epidermis

Tumor	Density of Langerhans Cells (×10⁻¹⁵)/μm²	
	Overlying Epidermis	Peritumoral Epidermis
1	11.26	12.99
2	6.8	28.45
3	23.94	32.44
4	3.91	12.16
5	9.81	20.89
6	18.71	26.42
7	11.61	13.21
8	8.61	20.45
9	12.52	24.30
10	18.32	33.86
11	15.09	16.50
12	15.33	37.52
Mean (SD)	12.99 (5.61)	23.27 (8.67)

Most research into the density of LCs in BCC is in the form of prospective studies of samples taken from frozen vertical sections[13-16] or epidermal sheets.[8,9] Both types of samples are useful for performing quantitative and structural studies of LCs.[21] The differences in epidermal LC densities in BCC have already been studied. Schreiner et al,[14] Azizi et al,[15] De Melo et al,[16] Bergfelt et al,[12] and McArdle et al[17] compared the densities of LCs in epidermal areas overlying the tumor with densities in perilesional epidermis or in contralateral healthy skin. However, the size of the epidermal area adjacent to the tumor or of healthy skin analyzed was not specified. Furthermore, other factors must be taken into consideration when studying LC density (especially in the case of comparative studies), eg, chronologic age, degree and type of UV radiation,[22,23] topographic location,[24] and methodology followed.[25]

We used formalin-fixed paraffin-embedded samples, which enabled us to perform a retrospective study, thus reducing the difficulty in obtaining the sample and the costs of processing. Similarly, when we compared the cellular density and morphology of LCs from 2 adjacent epidermal areas, bias stemming from factors such as chronologic age, topographic location, degree of exposure to UV radiation, and other immunosuppressive factors was minimized.

A number of studies have quantified LCs in BCC by comparing epidermal cell densities.[6,11-14,16,17] The epidermal area in these publications was determined by selecting the study field at random. In our opinion, this leads to a certain inaccuracy in measurement due to factors such as variation in the epidermal thickness of each area and reduction in LCs in the peritumoral epidermis with respect to the overlying area. In our study, we used image-processing technology to determine the overlying and peritumoral areas and the number of cells in the whole
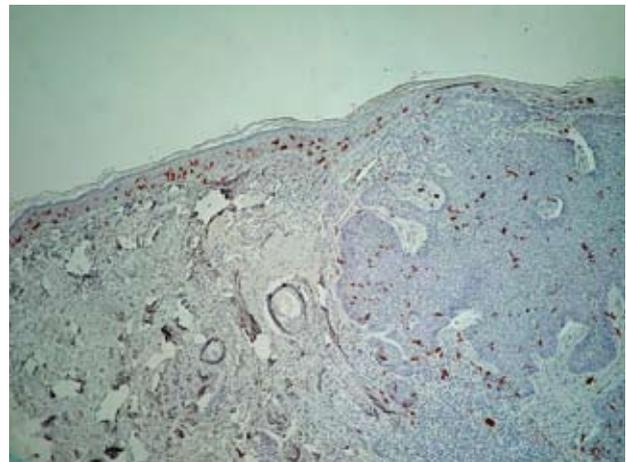

Figure 4. Staining of Langerhans cells in basal cell carcinoma (CD1a, ×40).

epidermal surface analyzed, thus providing us with a more accurate density of LCs per epidermal area. We observed fewer LCs in the overlying epidermis than in the peritumoral epidermis, which is consistent with the results of previous studies.[6,9,11-13] The lower density of CD1a+ LCs in the overlying area could be an indicator of migration of cells to regional lymph nodes or the loss of the ability to present antigens. This could result from the ability of LCs

exposed to UV radiation to migrate, apoptosis induced by UV radiation or tumor cells, or indeed from the loss of CD1a by LCs. In addition, the overlying epidermis was thinner than the peritumoral epidermis, which could enable UV radiation to have a greater effect on LCs. The lack of reduced LC density in the overlying epidermis in BCC observed by some authors[14,16,17] could be due to differences in histologic technique, cell markers, and time since onset of the tumor. Time since onset is noteworthy, since no studies have analyzed the relationship between LC density in the overlying epidermis of BCC and approximate time course or size of the tumor.

Optical microscopy revealed clear morphologic differences between CD1a+ LCs in the overlying areas and in peritumoral areas. Consistent with the results of Bergfelt et al[12] and Azizi et al[15], the cell bodies of the LCs in the overlying area took on a rounded or irregular appearance, with short and poorly branched dendrites. These findings could indicate dysfunctionality of LC due to the suppressive effect of tumor cells. Several aspects are interesting and worthy of evaluation: whether or not these morphologic changes are progressive in the development of the tumor, the association between these changes and the histologic type and aggressiveness of the tumor, and potential reversibility using imiquimod.

To conclude, the high prevalence and increased incidence of BCC has fostered the study of the mechanisms of development and biologic behavior of the tumor. Major advances have been made in our knowledge of the association between UV radiation, the cutaneous immune system, and BCC. Here, we aimed to expand upon the study of LCs in BCC by identifying LCs using an inexpensive, versatile, and reliable immunohistochemical technique. Optical microscopy and digital imaging technology also enabled us to confirm the usefulness of these techniques in a scientific study. This experience could be further developed in studies that expand knowledge on the pathogenic role and therapeutic potential of LCs in skin diseases.

Conflicts of Interest
The authors declare no conflicts of interest.

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