

NUMBER OF LANGERHANS CELLS IS DECREASED IN PREMALIGNANT KERATOSIS AND SKIN CANCERS

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Background: It was shown earlier that a number of CD207 positive Langerhans cells was lower in basal cell carcinomas than in the normal epidermis. Moreover, benign skin lesions presented a higher number of Langerhans cells when they were compared to malignant tumors. **Aim:** To count Langerhans cells, assessing expression levels of CD1A and CD207 markers in actinic keratosis, basal and squamous cell carcinomas, compared with the normal skin. Comparison of Langerhans cells might give a valuable prognostic marker for skin cancer. **Methods:** Immunohistochemistry and methods of statistics were used. **Results:** Expression of CD1A and CD207 markers was assessed in tumor samples of actinic keratosis, cutaneous basal and squamous cell carcinomas, in comparison with the normal skin. In each cohort there were 40 patients (and 11 healthy individuals). We have shown that the number of Langerhans cells is considerably lower in cutaneous basal and squamous cell carcinomas, compared with their number in the normal skin ($p < 0.0001$). **Conclusions:** CD1A expression correlated with CD207 expression only in the control group. There was no correlation in actinic keratosis, basal and squamous cell carcinoma. This may suggest an alteration of Langerhans cells phenotype in skin neoplastic diseases, making the number of Langerhans cells a valuable prognostic factor for skin tumors.

Key Words: Langerhans cell, CD207, CD1A, actinic keratosis, skin cancer.

Actinic keratosis is the most common precancerous sun-related lesion. The incidence of development of these lesions increases with age, proximity to the equator, and outdoor occupation [1]. Actinic keratosis has been recognized as a precursor of cancer and precancerous lesions in the past. However, today it is considered as an early *in situ* squamous cell carcinoma [2]. Histologically, there is cytological atypia in the basal/suprabasal layers of the epidermis. This lesion is characterized by the loss of orderly maturation with atypical keratinocytes. There are an increased number of mitoses. Parakeratosis and dyskeratosis are also common features with the usual appearance of elastosis in the dermis.

In the past two decades, a dramatic increase has been noted in the incidence of basal and squamous cell skin cancer [3]. Skin cancer is the most common cancer in Europe, United States, and Australia, and basal cell carcinoma accounts for approximately 80% of all skin cancers [4]. Basal cell carcinoma represents one of the most common cancers worldwide and is described as a locally invasive, slow-growing cancer which rarely metastasizes. Ultraviolet radiation is a major factor in non-melanoma skin cancer pathogenesis. The vast majority of basal cell carcinomas can be successfully treated by standard surgical excision before serious complications occur.

Squamous cell carcinoma is relatively common skin tumour which, if untreated, generally has a progressive clinical course with development of metastatic

disease and often a lethal outcome [5]. Histologically, lesions may differ in degree from actinic keratosis in that in squamous cell carcinoma *in situ* all epidermal layers contain atypical keratinocytes. Invasive squamous cell carcinoma is frequently derived from actinic keratosis [1].

Because skin lesions are visible and easily accessible, skin cancer provides us with an excellent model for studying the growth and development of cancer in humans. Furthermore, the carcinogenic agents in the skin are well known; in particular, ultraviolet radiation which is the most important carcinogen [6].

Langerhans cells are the dendritic cells of the epidermis that were discovered by Paul Langerhans in 1868 by gold chloride staining [7]. Langerhans cells comprise 3–8% of the total epidermal cell population [8]. Epidermal dendritic cells constitute a dense network located in the basal and supra-basal layers of the epidermis. At basal conditions, Langerhans cells are immature dendritic cells located between epithelial cells in the skin, nasal, oral, esophageal, pulmonary, vaginal, and rectal mucosae [9]. Epidermal dendritic cells have traditionally been defined by the presence of Birbeck granules. These racket-shaped intracytoplasmic organelles are only found in Langerhans cells. The function of Birbeck granules is still not completely understood, although most studies point toward an active role in receptor-mediated endocytosis through the C-type lectin (Langerin, CD207) [10]. Epidermal dendritic cells are distinguished from other dendritic cells subsets by expression of more specific markers, such as CD207 [10], E-cadherin [11] and CD1A [12]. These molecules are not only epidermal dendritic cells markers; they also play functional roles in various aspects of Langerhans cells biology.

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Abbreviations used: CD – cluster of differentiation; MHC – major histocompatibility complex.

CD1A (NP_001754) is a molecule belonging to the highly conserved group of CD1 transmembrane glycoproteins. The human CD1 family is structurally related to the major histocompatibility complex (MHC) proteins. It can form heterodimers with β_2 -microglobulin [13].

CD207 (Langerin, NP_056532) is a type II transmembrane protein. Its carbohydrate-recognition domain binds various monosaccharides, including fucose, mannose, and N-acetyl-glucosamin in a calcium-dependent manner [14]. CD207 is an endocytic receptor that functions as a potent inducer of Birbeck granules formation by the zipping and superimposition of cell membranes [10].

The CD207 protein is expressed in Langerhans cells. Mutations in CD207 gene resulted in inhibition of Birbeck granule formation that makes this molecule a valuable subject of study, especially in the skin cancers.

In the present work we report that the expression of CD1A and CD207 is lower, and number of Langerhans cells is considerably decreased in cutaneous basal and squamous cell carcinomas, compared with their number in the normal skin ($p < 0.0001$).

MATERIALS AND METHODS

Patient material. The study groups comprised 40 patients with actinic keratosis, cutaneous basal cell carcinoma and squamous cell carcinoma. The mean age of patients with actinic keratosis was 70.1 years (range 33–94) for a group of 21 women and 19 men; with cutaneous basal cell carcinoma — 71.2 years (range 46–94) for 22 women and 18 men; and for 24 women and 16 men with cutaneous squamous cell carcinoma — 74.3 years (range 50–94). The control group consisted of 11 patients (4 women, 7 men) aged from 47 to 92 years (mean 74.9). Patients included in the present study had neither systemic diseases nor any detected immunological abnormalities and did not take any preoperative medication.

Immunohistochemistry. Formalin fixed and paraffin embedded tissues (40 samples for each skin lesions and 11 samples for the control group) were retrieved from the archives. The 4 μ m thick sections were cut, mounted on slides and dried overnight at 55 °C. Paraffin was removed with xylene and the latter was washed away with ethanol. Endogenous peroxidase was blocked using a 3% solution of hydrogen peroxide in methanol. The monoclonal mouse antibodies against CD1A (clone 010) and CD207 (NCL-Langerin) were used to stain tissue sections (dilution 1:100, both antibodies from DakoCytomation, Denmark). Bound antibodies were visualized, using the Envision+Peroxidase Kit and the diaminobenzidine as a chromogen (DakoCytomation). The sections were counterstained with Mayer's haematoxylin. Stainings were examined with magnification ($\times 400$). A number of positive cells per 1000 cells were counted.

Statistical analysis. U-Mann — Whitney test, Kruskal — Wallis test, Spearman rank correlation test, and Wilcoxon test were used for statistical assess-

ments. P-values < 0.05 were considered statistically significant. Statistical analysis was performed, using Statistica version 8 for Windows.

RESULTS

Analysis of CD1A expression by immunohistochemistry. In order to assess an expression level of CD1A (NP_001754) and CD207 (NP_056532) proteins, tissue sections were stained with the appropriate antibodies. The CD1A positive cells among 1000 cells were counted. We have found that CD1A expression varied between the different neoplastic skin diseases, showing the mean of 1.0% in basal cell carcinomas, 2.2% in squamous cell carcinomas, 5.8% in actinic keratosis, and 4.8% in normal skin, respectively. The differences between tumors were statistically significant ($p < 0.0001$) (Table 1). The mean of CD1A expression in actinic keratosis was higher than the mean value for normal skin, however, this difference was not statistically significant ($p = 0.083$). A typical pattern of CD1A expression is shown on Fig. 1.

Table 1. CD1A expression in analyzed groups, scored as a percent per 1000 cells

Groups	Number	Min	Max	Mean	Standard deviation	Median
Basal cell carcinoma	40	0	6.0	1.0	1.0	1.0
Squamous cell carcinoma	40	0	4.0	2.2	1.0	2.0
Actinic keratosis	40	2.1	12.4	5.8	2.0	5.6
Control	11	3.0	8.7	4.8	1.7	4.0

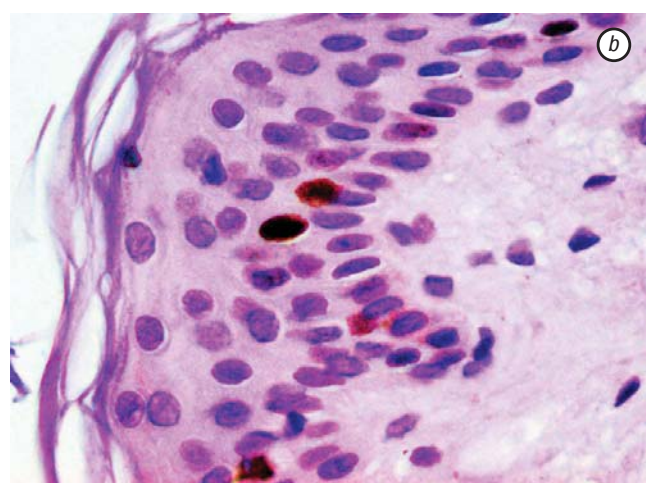
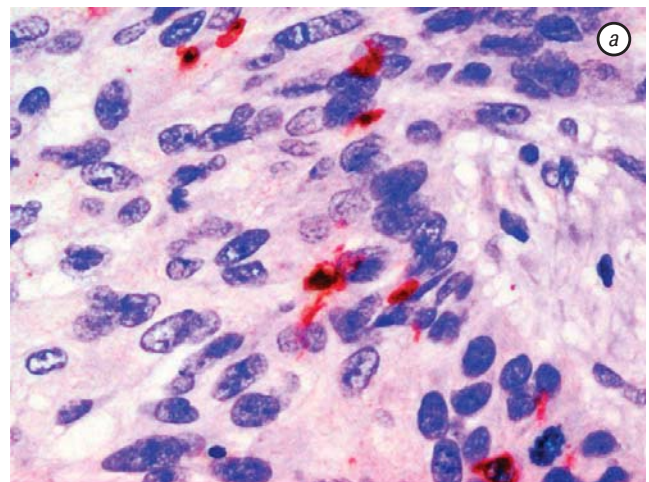


Fig. 1. A typical pattern of CD1A expression. Less cells showed CD1A signal (brown) in squamous cell carcinoma (a), compared with normal skin (b). Nuclei are shown in blue. Magnification was $\times 400$

Analysis of CD207 expression by immunohistochemistry. The same tissue sections were stained for CD207 in order to monitor the presence of Langerhans cells. The CD207 expression was lower significantly in all tumors studied, compared with normal skin (Table 2). In normal skin the CD207 signal was detected in 3.7% cells, compared with 2.9% in actinic keratosis, 0.8% in squamous cell carcinomas, and 0.5% in basal cell carcinomas, respectively ($p < 0.0001$). A representative CD207 staining is shown on Fig. 2.

Table 2. CD207 expression in analyzed groups, scored as a percent per 1000 cells

Groups	Number	Min	Max	Mean	Standard deviation	Median
Basal cell carcinoma	40	0	2.0	0.5	0.5	0.5
Squamous cell carcinoma	40	0	2.0	0.8	0.6	0.9
Actinic keratosis	40	1.0	6.7	2.9	1.0	3.0
Control	11	2.6	4.5	3.7	0.6	4.0

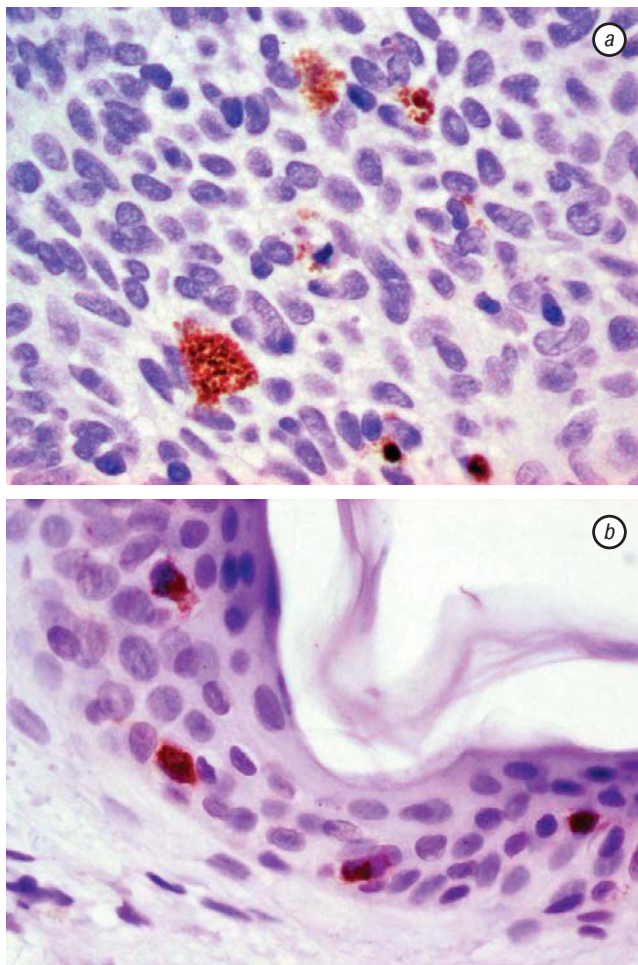


Fig. 2. A representative CD207 signal in tumor and normal tissues. Less cells showed CD207 signal (brown) in basal cell carcinoma (a), compared with normal skin (b). Nuclei are shown in blue. Magnification was $\times 400$

Statistical analysis of groups. After thorough analysis of the data, it was possible to conclude that CD1A signal was significantly higher in all groups of patients in comparison with the CD207 signal ($p < 0.05$) (Table 3).

A high value of CD1A signal followed a high CD207 only in the control group ($R = 0.652$; $p = 0.03$). This correlation was altered in a cases of basal cell carcinoma ($R = 0.252$; $p = 0.117$), squamous cell

carcinoma ($R = 0.06$; $p = 0.713$), and actinic keratosis ($R = 0.034$; $p = 0.835$).

Table 3. Comparison of expression of CD1A and CD207 in analyzed tumors compared with normal skin, scored as a percent per 1000 cells and presented as a mean value

Diagnosis	N	CD1A	CD207	p
Basal cell carcinoma	40	1.0	0.5	0.004
Squamous cell carcinoma	40	2.2	0.8	< 0.0001
Actinic keratosis	40	5.8	2.9	< 0.0001
Control	11	4.8	3.7	0.025

DISCUSSION

Based on our data it is possible to suggest that the number of Langerhans cells that usually express CD207 and also CD1A is considerably lower in cutaneous basal and squamous cell carcinomas, compared with their number in the normal skin. It was shown earlier that an incidence of CD207 positive Langerhans cells was lower in basal cell carcinomas as compared with the normal epidermis [15]. Moreover, benign skin lesions presented a higher number of Langerhans cells when they were compared to malignant and normal skin [16]. We have found similar phenomenon also for squamous cell carcinoma and, partially, for actinic keratosis. Notheworthy, the regressive neoplasm of the skin had the greatest dendritic cell infiltration compared to progressive neoplasm [17]. It was observed that such decrease in dendritic cell number could be a bad prognostic factor for other solid tumors as well. Actually, Langerhans cells density was proposed as a prognostic marker for laryngeal squamous cell carcinomas [18] and breast cancer [19]. Moreover, the lack of CD1A expression in the dendritic cells of Barrett's mataplasia may predict its evolution toward esophageal adenocarcinoma [20]; presence of CD1A expressing cells in tumors may influence metastasis [15].

In our study, the mean level of CD1A signal was significantly higher in all groups of patients than the level of CD207 expression ($p < 0.05$). This could be explained by the fact that precursors Langerhans cells lack CD207 positive Birbeck granules, the same can happen during the migration from the epidermis. Therefore, CD1A⁺ CD207⁻ cells could correspond to the replacement cells which have recently reached the epithelial compartment and are not yet expressing the CD207, or cells migrating toward the connective tissue, presenting with a down-regulation of the CD207 expression [21]. CD1A and CD207 molecules identify distinctive subpopulations of epithelial dendritic cells which express variable immunophenotypes that are influenced by the functional stage of these cells and the presence of various epithelial cytokines. Thus, distinct cytokines could be implicated in the induction of CD1A or CD207 expression.

CD1A expression correlated with CD207 expression only in the control group. There was no correlation in actinic keratosis, basal and squamous cell carcinoma; this may suggest an alteration of Langerhans cells phenotype in skin neoplastic diseases. Langerhans cells are considered to play an important role

in antitumour immunity. Vaccination with dendritic cells pulsed with tumour peptides, lysates or RNA, or loaded with apoptotic and necrotic tumour cells could induce significant antitumour immunity [22]. In the future, biomedical researchers and vaccine developers will seek to translate further progress in the biology of cutaneous dendritic cells into therapeutic indications.

CONCLUSION

Based on our data it is possible to speculate that the number of Langerhans cells is considerably lower in cutaneous basal and squamous cell carcinomas, compared with their number in the normal skin. CD1A expression correlated with CD207 expression only in the control group. There was no correlation in actinic keratosis, basal and squamous cell carcinoma. This may suggest an alteration of Langerhans cells phenotype in skin neoplastic diseases, making the number of Langerhans cells a valuable prognostic factor in skin tumors.

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