ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LXIII, N 2, 25, 2008 SECTIO D DOI: 10.2478/v10079-008-0065-4

Department of Clinical Pathology, Medical University of Lublin

ZORYANA SHEVCHUK, ELŻBIETA KOROBOWICZ

Langerhans cells in premalignant and malignant skin diseases

The skin is the largest organ of the body and has many different functions. The epidermis as the most superficial layer of the skin forms the first barrier of protection from invasion of foreign substances into the body. The skin is an essential part of the immune system. Immune cells of the epidermis and dermis participate in the defence against pathogens, in particular through T lymphocytes in transit, tissue macrophages and a network of cutaneous dendritic cells (DCs), which are key for perception of danger and initiating both innate and adaptive protective responses (1). DCs are antigen-presenting cells derived from the hematopoietic stem cell (2). LCs are the DCs of the epidermis (3). Skin resident LCs are well characterized and regarded as typical immature DCs. The identification of antigen, and the subsequent activation and migration of LCs towards the draining lymph nodes are well-studied processes (4). It is quite remarkable that, as early as 1868, Paul Langerhans discovered the stellate-shaped epidermal cells that now bear his name (5). However, 100 years were subsequently needed to establish the fact that LCs are potent antigen-presenting cells appertaining to the leukocyte system (6). In parallel, the pioneering work of Steinman and Cohn leading to the identification of splenic DCs (7) formed the basis for connecting LCs to the emerging DCs system. One common feature of DCs is their amazing faculty to present protein-derived peptide antigens to naive T lymphocytes (8). LC constituents of the skin immune system, are a network of dendritic major histocompatibility complex (MHC) class II antigen-presenting cells involved in initiation of cutaneous immune responses (9). LCs are important for presentation tumour-associated antigens. Probably they are integral in host resistance against malignant neoplasms that emerge in the epidermis. Local or distant progression of a malignant tumour is critically important for further destiny of patients. It is influenced by many factors dependent on the biological behaviour of tumour cells. The question of failure of the immune defence, where antigen presentation is a key component, must also be assessed. Whether LCs in tumour act the same way as LCs in normal tissues or whether the tumours injure LCs phenotype and function remains unknown (10). LCs have been studied in a variety of cancers (11–16). However, despite the significant amount of investigation in the past few years, a function of LCs in skin tumours is only partially known.

Squamous cell carcinoma (SCC) is relatively common skin tumour which if untreated, generally has a progressive clinical course with development of metastatic disease and often lethal outcome (17). With invasive SCC, masses of malignant keratinocytes extend through the basement membrane demonstrating different levels of atypia ranging from well differentiated to anaplastic. Having crossed over into the dermis they can metastasize.

Actinic keratosis (AK), also known as solar or senile keratosis is by far the most common precancerous dermatosis and is sunrelated disease (18). This lesion usually seen in individuals who are fair-skinned and who have occupations or hobbies that result in excessive sun exposure. AK may develop into carcinoma *in situ* or invasive SCC. This, however, is a controversial subject, based

amongst other on the absence of a clear-cut distinction between AK and SCC (19). Histologically, there is cytological atypia in the basal/suprabasal layers. Parakeratosis, dyskeratosis and elastosis in the dermis are also common features for AK (20).

Specific marker CD1a is now known to distinguish LCs from other DCs subsets (21). The present study compares the presence of cells exhibiting CD1a in AK and SCC.

METHODS

Formalin fixed, paraffin wax embedded tissue (40 cases for each skin lesions) was retrieved from the archives at the Pathology Department of Medical University of Lublin, Poland. Sections, 4-micrometer thick were cut coated slides and dried overnight at 55°C, dewaxed in xylene and rehydrated through industrial methylated spirits. Endogenous peroxidase was blocked using 3% hydrogen peroxide in methanol. Sections, after deparaffinization, were routinely processed, inclusive of antigen retrival according to manufacturer's prescription for immunocytochemical demonstration of expression of LCs by staining for the CD1a antigen using the Monoclonal Mouse Anti-Human CD1a, clone 010 (dilution 1:100, DakoCytomation, Denmark). Bound antibody was visualised using the Dakocytomation Envision+Peroxidase Kit, diaminobenzidine was used as chromogen. Sections were lightly counterstained with Mayer's haematoxylin. Positive cells were counted per 1,000 cells. The sections were examined at high power (x40) and positive nuclear staining was counted per 1000 cells.

RESULTS

The results showed the range of CD1a expression of total area to be 0,60%-4,00% (mean 2.21%) in SCC; 2.10%–12.40% (mean 5.76%) in AK. The positive cells were not found in the one of the samples studied. The number of LCs as measured by CD1a expression was significantly higher in AKs then SCCs ($p<0.001$). Mann-Whitney U Test was used in our study (Table 1).

Table 1. CD1a expression in SCC and AK, scored per 100 cells, presented as mean (M), standard deviation (SD), range (min, max) and as median (Me)

CD1a	n	min	max	M	SD	Me	p
SCC	39	0.60	4.00	2.21	0.94	2.00	<0.001
AK	40	2.10	12.40	5.76	1.98	5.55	

Mann-Whitney U Test was used in our study

DISCUSSION

CD1a-positive DCs were observed in the epidermis and dermis, but we studied epidermal antigen-presenting cells. In premalignant skin LCs were irregularly spaced within the basal and suprabasal layers. In SCCs examined LCs were concentrated at the peripheral areas of neoplastic epithelium.

Comparing the occurrence of LCs in the AKs and SCCs demonstrated a very low incidence of these cells in SCCs. Similarly to earlier published data, the incidence of LCs in the tumour tissue was reduced (23). This can indicate the failure of the immune defence in skin tumour, such as antigen presentation is a key component of immune surveillance.

Reasons for failure of the immune system to struggle with cancer include reduction of tumour immune mechanisms, limited availability of tumour-specific antigens, and inefficiency to deliver tumour antigens in the right immunological context. One of the most important questions remains the

delivery of these tumour antigens in an effective way to the immune system of a cancer patient. DCs perform this task in normal conditions. LCs are sentinels of the immune system located at sites of antigen entry such as skin (24). LCs may also play a role in defence mechanisms against neo-antigens in skin tumours. Reduced number of LCs in SCCs can confirm this conception.

Difference in immune responses of premalignant and malignant skin diseases indicate possible differences in the immunogenicity between these skin diseases.

Acknowledgments. We thank Mariola Bigas for excellent technical assistance. We are also grateful to Robert Klepacz for performing the statistical analysis.

REFERENCES

1. Valladeau J., Saeland S.: Cutaneous dendritic cells. Seminars in immunology, 17, 273, 2005.
2. Wright-Browne V., McClain K. L. et al.: Physiology and pathophysiology of dendritic cells. Human Pathology, 28, 563, 1997.
3. Romani N., Holzmann S. et al.: Langerhans cells-dendritic cells of the epidermis. APMIS, 111, 725, 2003.
4. Romani N., Ratzinger G. et al.: Migration of dendritic cells into lymphatics-the Langerhans cell example: routes, regulation and relevance. Int. Rev. Cytol., 207, 237, 2001.
5. Langerhans P.: Uber die nerven der menschlichen haut. Virchows Arch. Path. Anat., 44, 325, 1868.
6. Silberberg I.: Apposition of mononuclear cells to Langerhans cells in contact allergic reactions. An ultrastructural study. Acta Derm. Venereol., 53, 1, 1973.
7. Steinman R. M., Cohn Z. A.: Identification of a novel cell type in peripheral lymphoid organs of mice. Morphology, quantitation, tissue distribution. J. Exp. Med., 137, 1142, 1973.
8. Banchereau J., Steiman R. M.: Dendritic cells and the control of immunity. Nature, 392, 245, 1998.
9. Streilein J. W., Bergstresser P. R.: Langerhans cells: antigen presenting cells of the epidermis Immunobiology, 168, 285, 1984.
10. Byrne S. N., Halliday G. M.: Phagocytosis by dendritic cells rather than MHC II high macrophages is associated with skin tumour regression. Int. J. Cancer, 106, 736, 2003.
11. Tsujitani S., Furukawa T. et al.: Langerhans cells and prognosis in patients with gastric carcinoma. Cancer, 59, 501, 1987.
12. Schroeder S., Schwarz W. et al.: Dendritic/Langerhans cells and prognosis in patients with papillary thyroid carcinomas. Am. J. Clin. Pathol., 89, 295, 1988.
13. Kurihara K., Hashimoto N.: The pathological significance of Langerhans cells in oral cancer. J. Oral Pathol., 14, 289, 1985.
14. Nomori H., Watanabe S., Nakajma T. et al.: Histiocytes in nasopharyngeal carcinoma in relation to prognosis. Cancer, 57, 100, 1986.
15. Smolle J., Kandewitz P. et al.: Significance of nonlymphoid (accessory) cells in malignant lymphomas and pseudolymphomas of the skin. Br. J. Dermatol., 113, 677, 1985.
16. Gallo O., Libonati G. A. et al.: Langerhans cells related to prognosis in patients with laryngeal carcinoma. Arch. Otoryngol. Head Neck Surg., 117, 1007, 1991.
17. Kwa R. E., Campana K., Moy R. L.: Biology of cutaneous squamous cell carcinoma. J. Am. Acad. Dermatol., 26,1, 1992.
18. Stratton S. P., Dorr R. T., Alberts D. S.: The state-of-the-art in chemoprevention of skin cancer. Eur. J. Cancer, 36, 1292, 2000.

19. Berner A.: Actinic keratosis and development of cutaneous squamous cell carcinoma. Tidsskr Nor Laegeforen. 125, 1653, 2005.
20. Fu W., Cockerell C. J.: The actinic (solar) keratosis: a 21st-century perspective. Arch. Dermatol., 139, 66, 2003.
21. Mizumoto N., Takashima A.: CD1a and langerin: acting as more than Langerhans cell markers. Journal of Clinical Investigation, 113, 658, 2004.
22. Plzak J., Holikova Z. et al.: The role of dendritic cells in the pharynx. Eur. Arch. Otorhinolaryngol., 260, 266, 2003.
23. Schreiner T., Lischka G., Schaumburg-Lever G.: Langerhans cells in skin tumours. Arch. Dermatol., 131, 1463, 1995.
24. Nestle F. O.: Dendritic cells vaccination for the treatment of skin cancer. Recent Results Cancer Res., 160, 165, 2002.

SUMMARY

The aim was To compare differences in the number of Langerhans' cells (LC) between actinic keratosis (AK) and squamous cell carcinoma (SCC) in an attempt to suggest understanding of the local anti-tumour immune response. Forty cases of AK and forty cases of SCC were retrieved from the archives. Sections, 4-micrometer thick, were cut from formalin fixed, paraffin wax embedded in each case and were immunostained with the Monoclonal Mouse Anti-CD1a, positive nuclear staining was counted per 1,000 cells. The number of LCs as measured by CD1a expression was significantly higher in AKs than SCCs. Reduced incidence of LCs in SCCs was suggested for failure to deliver tumour antigens in an effective way to the immune system of a cancer patient.

Komórki Langerhansa w rakach skóry i stanach przedrakowych

Celem pracy było porównanie ilości komórek Langerhansa (KL) w rogowaceniu słonecznym (RS) i raku płaskonabłonkowym (RP) skóry w celu poznania mechanizmu miejscowej obrony organizmu. Bloczki parafinowe pacjentów z rozpoznanymi RS i RP (po 40 każdej jednostki nozologicznej) odnaleziono w archiwum. Pokrojone z nich skrawki o grubości 4 mikrometrów były poddane barwieniu przy pomocy metod immunohistochemicznych z udziałem monoklonalnego przeciwciała anti-CD1a, ilość KL wyliczano z liczby około 1000 komórek. Ilość KL oznaczona przez ekspresję CD1a była znacznie wyższa w RS w porównaniu z RP. Zredukowana ilość komórek Langerhansa w RP skóry może sugerować niepowodzenie prezentacji neoantygenów w układzie immunologicznym chorych.