Do Langerhans cells play a role in vulvar epithelium resistance to squamous cell carcinoma?

Helena Rotsztejn1, Ewa Trznadel-Budźko2 and Dorota Jesionek-Kupnicka3

1 Section of Dermatology, Research Institute of the Polish Mother’s Memorial Hospital, Łódź, Poland
2 Department of Dermatology and Pediatric Dermatology, Military-Medical Faculty, Medical University of Łódź, Poland
3 Department of Tumor Pathology, Chair of Oncology, Medical University of Łódź, Poland

Received: 2006.04.27, Accepted: 2006.07.17, Published online first: 2007.03.20

Abstract

Introduction: Langerhans cells (LCs) are a very important part of the skin immune system.

Materials and Methods: Skin biopsies taken from 13 women after the removal of vulvar squamous cell carcinoma (SCC) who had not been treated earlier for any vulvar diseases were investigated. The control group consisted of 12 women who underwent a plastic surgical operation of the vulva region. Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tissues samples using antihuman CD1a antibody (NCL-CD1a-235, Novocastra).

Results: This study showed a large decrease in LCs in vulvar SCC.

Conclusions: It is postulated that the reduction in the number of LCs may be one of the reasons for a higher tendency of carcinogenesis in the vulvar region. Their role as a main element of the skin immune system in the initiation of this process needs further investigation. It is possible that research on LCs in the skin will cast a new light on their role and even contribute to the prophylaxis and treatment of skin and mucosa carcinomas.

Key words: vulva, Langerhans cells, squamous cell carcinoma.

Corresponding author: Helena Rotsztejn, Ph.D. M.D., Section of Dermatology, Research Institute of the Polish Mother’s Memorial Hospital, Rzgowska 281/284, 93-338 Łódź, Poland, tel.: +48 42 271-10-00, e-mail: rotsztejn@onet.eu

INTRODUCTION

Langerhans cells (LCs) constitute 3–8% of all epithelial cells. The largest number is found in the basal and squamous layers. They are not present in the cornaceous layer. LCs are dendritic cells and they specialize in antigen presentation [14, 16]. Researchers have recently turned their attention to the relationship between LCs and carcinoma transformations in the skin. In the past, LCs were determined by means of the reaction with S-100 antigen. It turned out that this antigen was also present on other cells, so the results of the examinations were not precise, and even contradictory [1, 13]. At present, CD1a molecule and the positive reaction with the above-mentioned glycoprotein are basic to the evaluation of LCs [6]. Authors have also reported a diminishing and impairment of LC function as a result of immunosuppressive treatment, including applied locally corticosteroids and excessive UV radiation, especially UVB, which can subsequently lead to the development of skin carcinoma [15, 23]. It is proposed that LCs play a role in host resistance to malignant neoplasmas in the epidermis [8]. The aim of this study was to evaluate LCs in vulvar squamous cell carcinoma (SCC).

MATERIALS AND METHODS

The study group

We investigated skin biopsies taken from 13 women with vulvar SCC who had not been treated for any vulvar diseases earlier (mean age: 61.1±11.7 years). The patients underwent vulvectomy. The cases of SCC represented a well and moderate histopathological grade, with characteristic foci of keratinization. The adjacent tissues did not show pathological changes. The control group consisted of normal vulvar skin tissue obtained from surgical specimens from 12 women (mean age:...
who underwent a plastic surgical operation of the vulva region. The regional ethics committee approved the project. All enrolled subjects were informed about the purposes and methods of the research and gave their written consent.

**Immunohistochemistry**

Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tissues samples using antihuman CD1a antibody (NCL-CD1a-235, Novocastra). This antibody recognizes the human CD1a cell surface glycoprotein, a 43–49 kDa molecule expressed in association with β2 microglobulin. CD1a is expressed strongly by cortical thymocytes, LCs in the epidermis, dendritic cells in dermis, and also by LCs of mucosa of the tonsil.

The paraffin-embedded sections of tumor were deparaffinized, rehydrated, and heat-treated for citrate microwave antigen retrieval in 10 mM citrate buffer, pH 6.0, at 350 W for 15 min and then cooled to room temperature. Sections were then blocked for peroxidases in 0.3% H2O2 in methanol for 30 min, and incubated with the primary antibody anti-CD1a (dilution 1:50) for 30 min at room temperature in a humidity chamber. For detection, a Dako Envision System HRP with DAB staining and hematoxylin counterstaining was used. For a negative control, the slides were stained with the omission of the primary antibody. For a positive control, tissues of mucosa of the tonsil were used.

**Evaluation of LCs**

Immunohistochemical staining revealed that LCs exhibited characteristic processes. The number of LCs in each of the cases was estimated as the number of cells calculated per 10 high-power fields (HPFs) at 400× magnification.

**Statistics**

The examined group and the control group were compared by the Mann-Whitney U-test.

**RESULTS**

Immunohistochemical staining revealed that LCs exhibited cytoplasmatic characteristic processes. The number of LCs in SCC was very low compared with the control group (p<0.001; Table 1), the mean number of LCs being 0.85/10 HPFs (range: 0.0–2.0; Fig. 1). The mean number of LCs in the control tissue was 11.58/10 HPFs (range: 6.0–15.0; Fig. 2).

**DISCUSSION**

This study suggests that the decreased number of LCs may be directly connected with the process of carcinogenesis in the vulvar regions. The vulvar mucosa can...
be a starting point for SCC. In this region, carcinogene-
sis seems to be connected with infections and inflamma-
tion processes, which in turn may be caused by mechan-
ical traumas (9). This may cause impairment of the
skin’s immune system, which can lead to carcinogenic
transformation. Moreover, it is worth underlining that
in vulvar lichen sclerosus, oxidative stress plays a signifi-
cant role in involved tissue. Oxidative damage to lipids,
DNA, and proteins may contribute to oxidative tissue
injury, which may also lead to carcinogenesis [20].
Nitrative damage to nucleic acids also plays an impor-
tant role in carcinogenesis. This occurs during inflam-
matory processes when reactive nitrogen species, such as
peroxynitrite, nitroxyl, and nitrogen dioxide, are gen-
erated. This may lead to the accumulation of mutagenic
DNA lesions and p53 in damaged epithelium and carcino-
ma connected with inflammation [4, 12].

The connection between inflammation and cancer
caused by chronic infection has been recognized in
numerous solid tumors. *Helicobacter pylori*-induced gas-
tritis leading to gastric cancer, inflammatory bowel dis-
ease leading to colorectal cancer, inflammation in the
pancreas, chronic viral hepatitis leading to liver cancer,
and oral lichen planus leading to squamous oral cancer
are among the examples [4, 5, 7, 12].

It is believed that vulvar SCC may be divided into
two groups: a group originating from vulvar intraepithe-
elial neoplasia, in which human papilloma virus (HPV)
infection may cause carcinogenesis, and an SCC group
which is connected with chronic vulvar dystrophy [3, 17].
Various dystrophic lesions of the vulvar epithelium were
found in about 70–80% of SCC surrounding tissues [19].

Some authors also raise a question of the long-term
application of steroid ointments in vulvar inflammation
processes and the possibility of it leading to latent HPV
infection. This may indicate that vulvar dystrophy is a
process which can lead to carcinoma development.
Other authors divide vulvar SCC into HPV-dependent
and HPV-independent [6, 18, 21].

Many investigations have proved that UV radiation,
especially UVB, has a similar influence on decreasing
the LC number in the skin in a dose-dependent manner
[22]. Interestingly, some studies also exhibited morpho-
logical changes of LCs (deformation of the dendritic
processes and alterations of the Birbeck granules) [1, 24].
The immunosuppressive activity of UV radiation is
caused by cytokines secreted by epithelial cells, mainly
interleukin IL-10. This influences the skin’s immune sys-
tem through lowering the secretion of other cytokines
and inhibiting antigen presentation by LCs. At the same
time, IL-10 influences LCs, transforming them into
inactive forms [2, 11].

In patients who underwent renal transplantation,
ipairment of the immune system function was con-
ected with the development of skin carcinoma. An
increased frequency of skin carcinoma, mainly SCC, was
found in patients after renal transplantation subjected
to immunosuppression [10].

Our findings clearly show a large decrease in or
absence of LCs in vulvar SCC. The number of LCs was
significantly decreased in the epidermis in Bowen’s dis-
ease [6]. Investigations indicate a decrease in LC num-
ber in the epithelium of the inner surface of the pre-
puce, which, according to the researchers, together with
infections and increased incidence of traumas in this
region, may trigger carcinogenesis [25].

In SCC, very few CD1-positive LCs were concen-
trated at the periphery of neoplastic epithelium, while
they were absent in the central part of the tumor [24].

The role of LCs as a main element of the
skin’s immune system in the initiation of the carcino-
genesis process needs further investigation. It is possible
that research on LCs in the skin will cast a new light on
their role and even contribute to the prophylaxis and
treatment of skin and mucosa carcinomas.

**REFERENCES**

1. Alcalay J., Goldberg L. H., Wolf J. E. Jr. and Kripke M. L. (1989): Variations in the number and morphology of
Langerhans’ cells in the epidermal component of squa-
mous cell carcinomas. Arch. Dermatol., 125, 917–920.

2. Asadullah K., Docke W. D., Sabat R., Ebeling W., Volk H. D. and Sterry W. (1999): Interleukin-10 in der
Hautartzt, 50, 12–19.

3. Carlson J. A., Ambros R., Malfeitano J., Ross J., Grab-
owsk R., Lamb P., Figge H. and Mihm M. C. Jr. (1998):
Vulvar lichen sclerosus and squamous cell carcinoma:
a cohort, case control, and investigational study with his-
torical perspective; implications for chronic inflamma-
tion and sclerosis in the development of neoplasia. Hum.
Pathol., 29, 932–948.

4. Chaiyarat P., Ma N., Hiraku Y., Pinlaor S., Yongvanit P.,
Jintakanon P., Murata M., Oikawa S. and Kawanish S. (2005): Nitrative and oxidative DNA damage in oral lichen
planus in relation to human oral carcinogenesis. Cancer
Sci., 96, 553–559.

5. Crowe S. E. (2005): Helicobacter infection, chronic inflam-
mation, and the development of malignancy. Curr. Opin.
Gastroenterol., 21, 32–38.

6. Duan H., Koga T., Masuda T., Mashino T., Imafuku S.,
Terao H., Murakami Y., Urabe K., Kiryu H. and Furue M. (2000): CD1a+, CD3+, CD4+, CD8+, CD68+ and cuta-
neous lymphocyte-associated antigen-positive cells in
Bowen’s disease. Br. J. Dermatol., 143, 1211–1216.

7. Farrow B. and Evers B. M. (2002): Inflammation and the
development of pancreatic cancer. Surg. Oncol., 10, 153–169.

8. Fernandez-Bussay R., Cambazdar F., Mauduit G. Schmitt D.
and Thivolet J. (1983): T cell subsets and Langerhans
cells in skin tumours. Eur. J. Cancer Clin. Oncol., 19,
907–913.

9. Fox H. and Wells M. (2003): Recent advances in the
pathology of the vulva. Histopathology, 43, 209–216.

10. Galvao M. M., Sotto M. N., Kihara S. M. Rivitti E. A. and
Sabbaga E. (1998): Lymphocyte subsets and Langerhans
cells in sun-protected and sun exposed skin of immuno-
suppressed renal allograft recipients. J. Am. Acad.
Dermatol., 38, 38–44.

11. Halliday G. M. and Le S. (2001): Transforming growth fac-
tor-beta produced by regressor tumors inhibits, while IL-
-10 produced by regressor tumors enhances, Langerhans
cell migration from skin. Int. Immunol., 13, 1147–1154.
12. Itzkowitz S. H. and Yio X. (2004): Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. Am. J. Physiol. Gastrointest. Liver Physiol., 287, G7–17.
13. Kurihara K. and Hashimoto N. (1985): The pathological significance of Langerhans cells oral cancer. J. Oral Pathol., 14, 289–298.
14. Meunier L. (1996): Immune dendritic cells in human dermis. Eur. J. Dermatol., 6, 327–331.
15. Meunier L. (1999): Ultraviolet light and dendritic cells. Eur. J. Dermatol., 9, 269–275.
16. Nakagawa S. and Bost J. D. (2001): Role of Langerhans cells in the skin. What’s new? J. Eur. Acad. Dermatol. Venereol., 15, 399–401.
17. Poulsen H., Junge J., Vyberg M., Horn T. and Lundvall F. (2003): Small vulvar squamous cell carcinomas and adjacent tissues. A morphologic study. APMIS, 111, 835–842.
18. Riethdorf S., Neffen E. F., Cviko A., Loning T., Crum C. P. and Riethdorf L. (2004): p16INK4A expression as biomarker for HPV 16-related vulvar neoplasia. Hum. Pathol., 35, 1477–1483.
19. Rouzier R., Morice P., Haie-Meder C., Lhomme C., Avril M. F., Duvillard P. and Castaigne D. (2001): Prognostic significance of epithelial disorders adjacent to invasive vulvar carcinomas. Gynecol. Oncol., 81, 414–419.
20. Sander C. S., Ali I., Dean D., Thiele J. J. and Wojnarowska F. (2004): Oxidative stress is implicated in the pathogenesis of lichen sclerosus. Br. J. Dermatol., 151, 627–635.
21. Scurry J. P. and Vanin K. (1997): Vulvar squamous cell carcinoma and lichen sclerosus. Australas. J. Dermatol., 38 (suppl. 1), 20–25.
22. Seite S., Zucchi H., Moyal D., Tison S., Compan D., Christiaens F., Gueniche A. and Fourtanier A. (2003): Alterations in human epidermal Langerhans cells by ultraviolet radiation: quantitative and morphological study. Br. J. Dermatol., 148, 291–299.
23. Tiplica G. S. (2001): Dermatocorticoterapie. Curtea Veche, Bukaresti, 20–21.
24. Townsend W. L., Gorrell M. D. and Mayer R. (1997): Langerhans cells in the development of skin cancer: a qualitative and quantitative comparison of cell markers in normal, acanthotic and neoplastic ovine skin. Pathology, 29, 42–50.
25. Weiss G. N., Sanders M. and Westbrook K. C. (1993): The distribution and density of langerhans cells in the human prepuce: site of a diminished immune response? Isr. J. Med. Sci., 29, 42–43.