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Langerhans cells in lichen sclerosus of the vulva and lichen sclerosus evolving in vulvar squamous cell carcinoma

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Summary. Vulvar lichen sclerosus (LS) represents a benign chronic inflammatory skin lesion that carries a risk for development of vulvar squamous cell carcinoma (SCC). We aimed at determining whether premalignant changes in vulvar LS, a multifactorial disease, presenting a welter of evidence implicating the immune system in its pathogenesis, could be identified by analysing the Langerhans' cells (LCs), the primary cell responsible for antigen recognition and presentation.

The relationship existing between inflammation and cancer due to chronic infection, and demonstrated in many solid tumors, led us to study LCs in eight cases of vulvar LS, which showed an evolution to carcinoma of the vulva and in ten cases of unchanged vulvar LS in matched patients by immunohistochemistry for antibodies CD1a and S100. We did not find a statistically significantly different number of LCs counted either in S100 stained specimens, nor in CD1a stained specimens of LS epithelium in unchanged or evolving cases. The data emerging in our study do not support the hypothesis that the variation in the number of LCs may be related to the development of SCC in late stage LS cases.

Key words: Lichen sclerosus, Vulva, Langerhans cells, Squamous cell carcinoma

Introduction

Lichen sclerosus (LS) is a chronic, sclerosing, lymphocyte-mediates skin disease and occurs predominantly in genital skin (Powell et al., 1999) LS can be diagnosed in young women and even in prepubertal girls (Powell et al., 2001), but the majority of LS occurs in vulvar skin of postmenopausal women.

LS represents a benign chronic inflammatory skin lesion; however, vulvar LS carries an increased risk for development of vulvar squamous cell carcinoma (SCC) (Carli et al., 1995). In addition, histo-pathologic analysis of surgical specimens of SCC reports LS in the skin near cancer (Hart et al., 1975; Carlson et al., 1998).

Invasive SCC of the vulva is currently classified into three histologic sub-types: basaloid, warty, and keratinizing (Kurman et al., 1993). The first and the second sub-histotypes, strongly related to high-risk human papilomavirus (HPV), occur in young women, and there is a high risk of association with squamous neoplasia elsewhere in the lower genital tract (Mitchell et al., 1993; Al-Gramdi et al., 2002).

Conversely, the keratinizing type, the most frequent one, is not HPV correlated; it occurs in post-menopausal women, and it is often reported as a large tumor when initially diagnosed (Rutledge et al., 1991).

Histologically, LS is characterized by hyper-keratosis, thin epidermis and/or loss of rete ridges, vacuolar interface changes, edema and/or sclerosis of dermal collagen and chronic inflammatory cell infiltrate of dermis (Chiesa-Vottero et al., 2006). The lymphocytic infiltrate is characterized by a predominant T-cell phenotype (Regauer et al., 2002).

The etiology of vulvar LS is unknown, but as happens in other infiammatory diseases (Takeuchi et al., 1988) the recruitment of lymphocytes to the skin plays a significant role in the evolution of vulvar LS.

Within the squamous epithelium, the Langerhans' cells (LCs) have been shown to be the primary cells responsible for antigen recognition and presentation, making this specialized tissue macrophage of key importance in the local immune defense of skin and mucosae, such as vulvar epithelium (Meunier, 1999; Nakagawa and Bost, 2001).

The largest number of LCs is found in the basal and

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squamous layers (Lipozenic and Ljubojevic, 2004).

LCs play an important role in the skin immune system, being part of a wide system of dendritic cells specialized in antigen presentation (Steinman, 1991). LCs can migrate to regional lymph nodes and present antigens to naïve T cells; activated T cells can migrate to the skin, resulting in inflammatory infiltrates and skin disease. For this reason, attention has been directed to the study of LCs, which constitutes a primordial step in local immune response involving T cells. It is reported that reduction in LC population could be associated with an increased enhancement of epithelial apoptosis in HIV infected women (Walker et al., 2005). It would be expected that alterations in the population of LC could provoke a deficiency in the generation of an adequate immune response, which would greatly facilitate the persistence of a chronic inflammatory state, transforming the epithelial tissue into proper terrain for cancer (Al-Saleh et al,, 1995).

The relationship existing between inflammation and cancer due to chronic infection which has been demonstrated in many solid tumors, such as inflammatory bowel diseases with colorectal carcinoma, (Baumgart and Sandborn, 2007) or chronic viral hepatitis with liver carcinoma, (Goodman, 2007) led us to better study the LCs in a series of late vulvar LS cases with different clinical evolution. Our aim was to evaluate quantitatively LCs in late vulvar LS and to demonstrate a variation, if any, in the count of LCs related to the development of SCC in late stage LS cases. For this purpose we counted LCs in the epithelium of vulvar LS by immunohistochemistry for antibodies CD1a and S100, each targeting a different element of the LCs. CD1a is a protein of 43 to 49 kD and has been shown to be expressed on dendritic cells and cortical thymocytes. Langerhans cells in the skin and some epithelia also express this protein.

We tested the two antibodies within the same specimen according to the results of previous studies reporting that the use of the S100 antibody immunostaining technique tends to demonstrate low LCs counts, while those that use monoclonal antibody anti CD1may allow to show a reduced, normal, or even increased number of LCs (Tay et al., 1987; Caorsi and Figueroa, 1986; Vayyryen et al., 1984).

These experimental data lead us to postulate that a defect in the production or function of the S100 protein in the LCs, not a decrease in their actual number, may lead to a defect in antigen presentation that allows malignant transformation to elude the immune system (Connor et al., 1999).

Materials and methods

Patients

Among 148 vulvar LS cases documented by vulvar biopsy collected in the files of the Department of Human Pathology and Oncology of the University of Florence from 1995 to 2004, we identified eight cases of vulvar LS, which showed an evolution to vulvar SCC, histologically documented.

The slides and tissue blocks of all eight biopsies with diagnosis of LS and the eight surgical specimens with diagnosis of SCC were reviewed to confirm the histologic diagnosis.

All cases were longstanding LS characterized by atrophic epidermis, sclerosis of dermal collagen, with moderate or scant lichenoid aggregate, lymphocytic aggregates (LA), either around blood vessels or hair appendages in the deep submucosa or deeply extending biopsies.

The histological type of each SCC was determined according to the criteria of Kurman et al. (1993). All eight squamous cell carcinomas were of the keratinizing histotype and LS was observed in the skin near cancer.

The mean age of the patients with vulvar LS was 61.3 years (range, 56 to 79 years). The patients evolved to vulvar SCC after a period ranging from 10 months to 9 years.

We also selected, as controls, ten vulvar LS of matched patients, for whom follow-up information was available for a minimum of ten years.

Therefore, we evaluated the histological features, the immunohistochemical staining, of two groups of LS cases: LS unchanged and LS evolved to SCC.

Tissue specimens and immunohistochemistry

Hematoxylin-eosin stained sections from each histological specimen were reviewed by two pathologists (GLT, MRR) to confirm the histological diagnosis. We selected one representative paraffin block from each case for further studies.

A representative section for each specimen was selected for immunohistochemical analysis with S100 and CD1a staining, respectively.

All sections were deparaffinized in Bio-Clear (Bio-Optica, Milan, Italy) and hydrated with graded ethanol concentrations until distilled water; they were them placed in 3% hydrogen peroxide to block endogenous peroxidase. The primary antibody S100 Polyclonal rabbit anti-Cow (Dako, Carpenteria,CA) was used at dilution 1:2000. This antibody reacts strongly with human S100B, and weakly or very weakly with S100A1 and S100A6, respectively. All tissue sections were placed on the Ventana Nexes automated stainer using as rivelation system iVIEW DAB Detection Kit (Ventana Medical Systems, Tucson, Arizona) and incubated for 32 minutes at 37°C. The negative control was included with each run by substituting the primary antibody with non immune rabbit serum; a cutaneous nevus was used as positive control. The control sections were treated in parallel with the samples in the same run. Subsequently, with other sections of the same specimens, we weighed monoclonal antibody CD1a (Ventana Medical System) ready-to-use.

All tissue sections were then placed on the Ventana

automated stainer BenchMark XT™ ICH system using the rivelation system iVIEW DAB. Primary antibody anti-CD1a was placed on the tissue sections and incubated for 32 minutes at 37°C.

A negative control sample was included with each run by omitting the primary antibody and yielded no signal. A positive control was skin. The control sections were treated in parallel with the samples in the same run. After the staining run was complete, the tissue sections were removed from the two stainers, counterstained with hematoxylin, dehydrated and mounted with Permount.

Evaluation of LC

Immunohistochemical staining revealed that the LCs exhibited characteristic processes. The reaction was considered positive for antibodies S100 and CD1a when the cytoplasm of cells with their cell processes became stained in brown color. The number of LCs in each case was estimated as the number of cells calculated per 10 high-power fields (HPF) at 400x magnification, blind to the clinical details and identity of patients, as previously described (Rotszetejn et al., 2006).

Statistical analysis

The difference in number of LCs with S100 and CD1a positive staining in the two groups of LS cases (evolving to vulvar SCC and unchanged) were calculated according to the Wilcoxon Mann-Whitney test (Fisher and Van Belle, 1993). Data analysis was performed using the SPSS Version 14.1 (Chicago, IL) statistical package. A P value ≤ 0.05 was considered to be statistically significant.

Results

Histopathologic features

The analyzed samples were of longstanding LS with atrophic epidermis with sclerosis of dermal collagen, with scant lichenoid aggregate (Fig. 1). However, these biopsies often showed lymphocytic aggregates (LA), either around blood vessels or hair appendages in the deep submucosa or deeply extending biopsies.

Immunohistochemistry

Immunohistochemical staining revealed that LCs exhibited cytoplasmatic characteristic processes both with S100 and CD1a staining (Fig. 2). The mean number (±SD) of LCs S100 positive was 1.2 (±0.3) per 10 high-power fields (HPF) at 400x magnification in LS cases evolving to SCC, while it was 1.3 (±0.3) per 10 high-power fields (HPF) at 400x magnification in not evolving cases (P value not significant). The mean

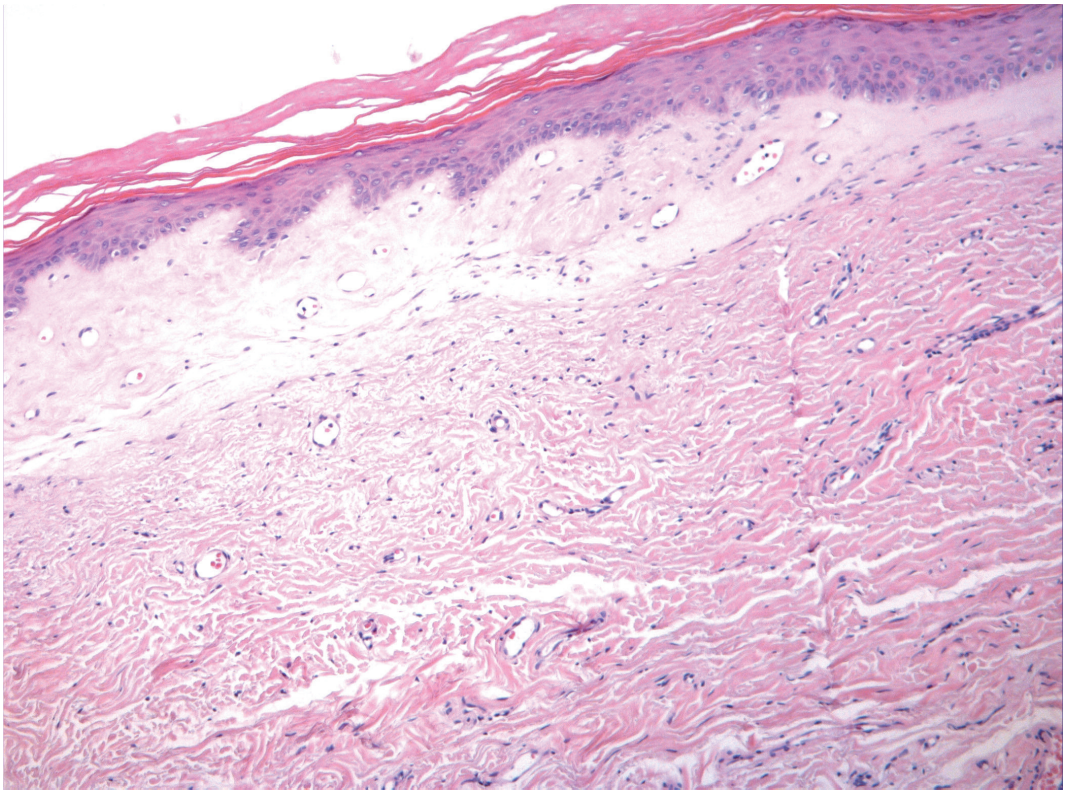

Fig. 1. Hematoxilin-eosin staining showing a longstanding LS with atrophic epidermis, loss of rete ridges, edema and sclerosis of dermal collagen, with scant chronic inflammatory infiltrates. x 20.

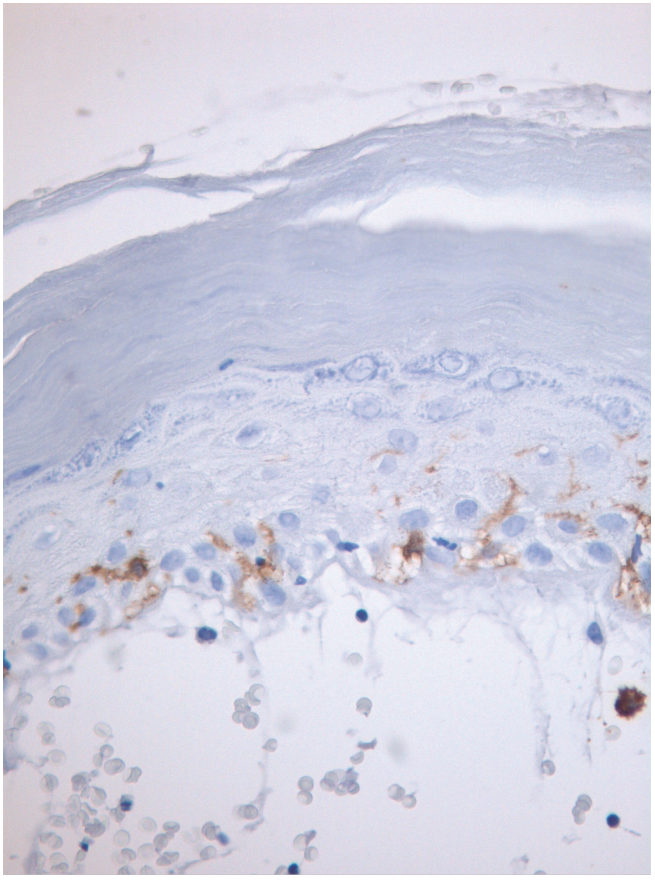

Fig. 2. CD1a positive immunohistochemical staining in LC exhibing cytoplasmatic characteristic processes. x 40.

number (±SD) of LCs CD1a positive was 8 (±1) per 10 high-power fields (HPF) at 400x magnification in LS cases evolving to SCC, while it was 7.2 (±1.1) per 10 high-power fields (HPF) at 400x magnification in not evolving cases (P value not significant).

Discussion

To the best of our knowledge, this is the first study reporting the count of LCs in vulvar LS cases in comparison with LS evolving to vulvar SCC. We did not find a statistically significantly different number of LCs counted either in S100 stained specimens, nor in CD1a stained specimens of LS epithelium in unchanged or evolving cases.

Different experimental and literature data support a relationship between vulvar LS and SCC: in the specimens excised for vulvar SCC the presence of LS is reported (Scurry and Vanin, 1997; Carli et al,, 2003), and the incidence of SCC is higher in women presenting LS in comparison to women without this vulvar disease (Carli et al., 1995; Jones et al., 2004). The etiology of LS is believed to be multifactorial, so it makes difficult the search for a marker of malignancy. We previously observed that the evaluation of MIB1 labelling index (Raspollini et al., 2007a) and the study of markers of angiogenesis (Raspollini et al., 2007b) may identify those cases of vulvar LS which most likely evolve to SCC, therefore requiring careful periodic checks or adjunctive biopsies. However, while there is still debate about whether LS is a primary connective tissue pathology or an immunological disorder, there is a welter of evidence implicating the immune system in its pathogenesis (Barnes and Douglas, 1985; Scurry, 1999). In fact, it has been observed a predominant T-lymphocytes infiltrate, increased cytokine expression and a good response to corticosteroids. Our recent data do not support a specific role of lymphocytic infiltrate in vulvar carcinogenesis (Raspollini et al., 2008). However, molecular assessement of lymphocytic infiltrate reporting a low percentage of clonal T cells in LS strongly supports a chronic local antigen esposure; therefore the skin immune system seems to be involved in the disease (Regauer and Beham-Schmid, 2006). The recruitment of lymphocytes to the skin may also be connected with the recruitment of LCs to the skin. Rotszetein et al. (2006) observed that in early LS lesions there are more LCs than within late LS cases. This difference in number may be connected with the IL-1 production by keratinocites; (Schon and Ludwig, 2005) and the intensity of lymphocyte infiltrates is modified between early and late stage of LS. Rotszetein et al. (2007) also observed that LCs in vulvar SCC are remarkably diminished, leading to hypothesize that a reduction in the number of LCs may be one of the reasons for a higher tendency to carcinogenesis in vulvar LS cases. The data emerging in our study do not support the hypothesis that the variation in the count of LCs is related to the development of SCC in late stage LS cases. Both the count performed in S100 and CD1a positive stained specimens show a similar LC number in the evolving and in the unchanged late stage vulvar LS cases. Carcinogenesis is a multistep process in which many events occur. Recent data have expanded the concept of inflammation also being a critical component of carcinogenesis (Marnett, 2000). Free radicals produced by chronic inflammation can induce several alterations, such as gene mutations and post-translational modifications of key cancer-related proteins. These modifications may lead to the disruption of cellular processes, including DNA repair and apoptosis (Coussens and Werb, 2002). Therefore, the chronic inflammatory processes observed in LS may contribute to the development of malignancies by oxidative processes (Sander et al., 2004). In damaged tissue, due to chronic inflammatory mechanism, such as the vulvar LS epithelium, the starting point, i.e., mutagenic DNA lesions or p53 mutations (Raspollini et al., 2007a) in damaged epithelium, may have an easy way for development of cancer.

References

Al-Gramdi A., Freedman D., Miller D., Poh C., Rosin M., Zhang L. and

Gilks C.B. (2002). Vulvar squamous cell carcinoma in young women: a clinicopathologic study of 21 cases. Gynecol. Oncol. 84, 94-101.

Al-Saleh W., Delvenne P., Arrese J.E., Nikkels A.F., Pierard G.E. and Boniver J. (1995). Inverse modulation of intraepithelial Lagerhans cells and stromal macrophage-dendrocyte populations in human papillomavirus-associated to squamous intraepithelial lesions of the cervix. Virchows Arch. 427, 41-48.

Barnes C.J. and Douglas C.P. (1985). Preliminary findings on levels of collagenase and its tissue inhibitor in some vulvar dystrophies. J. Obstet. Gynaecol. 6, 55-56.

Baumgart D.C. and Sandborn W.J. (2007). Inflammatory bowel disease: clinical aspects and established and evolving therapies. Lancet 369,1641-1657.

Caorsi I. and Figueroa C.D. (1986). Langerhans' cell density in the normal exocervical epithelium and in the cervical intraepithelial neoplasia. Br. J. Obstet. Gynaecol. 93, 993-998.

Carli P., Cattaneo A., De Magnis A., Biggeri A., Taddei G.L. and Giannotti B. (1995). Squamous cell carcinoma arising in vulval lichen sclerosus: a longitudinal cohort study. Eur. J. Cancer Prev. 4, 491-495.

Carli P., De Magnis A., Mannone F., Botti E., Taddei G. and Cattaneo A. (2003). Vulvar carcinoma associated with lichen sclerosus. Experience at the Florence, Italy, Vulvar Clinic. J. Reprod. Med. 48, 313-318.

Carlson J.A., Ambros R., Malfetano J., Ross J., Grabowski R., Lamb P., Figge H. and Mihm M.C. Jr. (1998). Vulvar lichen sclerosus and squamous cell carcinoma: a cohort, case control and investigation study with historically perspective; implications for chronic inflammation and sclerosis in the development of neoplasia. Hum. Pathol. 29, 932-948.

Chiesa-Vottero A., Dvoretsky P. and Hart W. (2006). Histopathologic study of thin vulvar squamous cell carcinomas and associated cutaneous lesions: a correlative study of 48 tumors in 44 patients with analysis of adjacent vulvar intraepithelial neoplasia types and lichen sclerosus. Am. J. Surg. Pathol. 30, 310-318.

Connor J.P., Ferrer K., Kane J.P. and Goldberg J.M. (1999). Evaluation of Langerhans' cells in the cervical epithelium of women with cervical intraepithelial neoplasia. Gynecol. Oncol. 75,130-135.

Coussens L.M. and Werb Z. (2002). Inflammation and cancer. Nature 420, 860-867.

Fisher L.D. and Van Belle G. (1993). Biostatistics: A methodology for the health sciences. 2nd edition, 3th serie. A Wiley Interscience Publication. London. pp 1-80.

Goodman Z.D. (2007). Neoplasms of the liver. Mod. Pathol. 20 Suppl 1, S49-S60.

Hart W.R., Norris N.J. and Helving E.B. (1975). Relation of lichen sclerosus et atrophicus of the vulva to development of carcinoma. Obstet. Gynecol. 45, 369-377.

Jones R.W., Sadler L., Grant S., Whineray J., Exeter M. and Rowan D. (2004). Clinically identifying women with vulvar lichen sclerosus at increased risk of squamous cell carcinoma. J. Reprod. Med. 49, 808-811.

Kurman R.J., Toki T. and Schiffman M.H. (1993). Basaloid and warty carcinomas of the vulva: distinctive types of squamous cell carcinoma frequently associated with human papillomaviruses. Am. J. Surg. Pathol. 1, 133-145.

Lipozenic J. and Ljubojevic S. (2004). Identification of Lagerhans cells in dermatology. Arch. Hig. Rada. Toksikol. 55, 167-174.

Marnett L.J. (2000). Oxyradicals and DNA damage. Carcinogenesis. 21, 361-370.

Meunier L. (1999). Immune dendritic cells in human dermis. Eur. J. Dermatol. 9,269-275.

Mitchell M.F., Prasad C.J., Silva E.G., Rutledge F.N., McArthur M.C. and Crum C.P. (1993). Second genital primary squamous neoplasms in vulvar carcinoma: viral and histopathologic correlates. Obstet. Gynecol. 81,13-18.

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.

Powell J.J. and Wojnarowska F. (1999). Lichen sclerosus. Lancet 353, 1777-1783.

Powell J.J. and Wojnarowska F. (2001). Childhood vulvar lichen sclerosus: an increasingly common problem. J. Am. Acad. Dermatol. 44, 803-806.

Raspollini M.R., Asirelli G., Moncini D. and Taddei G.L. (2007a). A comparative analysis of lichen sclerosus of the vulva and lichen sclerosus that evolves in vulvar squamous cell carcinoma. Am. J. Obstet. Gynecol. 197, 592. e1-5.

Raspollini M.R., Asirelli G. and Taddei G.L. (2007b). The role of angiogenesis and COX-2 expression in the evolution of vulvar lichen sclerosus to squamous cell carcinoma of the vulva. Gynecol. Oncol. 106, 567-571

Raspollini M.R., Asirelli G. and Taddei G.L. (2008). Analysis of lymphocytic infiltrate does not help in prediction of the vulvar squamous cell carcinoma arising in a background of lichen sclerosus. Int. J. Gynecol. Obstet. 100, 190-191.

Regauer S. and Beham-Schmid C. (2006). Detailed analysis of the T-cell lymphocytic infiltrate in penile lichen sclerosus an innumohistochemical and molecular investigation. Hystopathology. 48, 730-735.

Regauer S., Reich O. and Beham-Schmid C. (2002). Monoclonal gamma-T-cell receptor rearrangement in vulvar lichen sclerosus and squamous cell carcinomas. Am. J. Pathol. 160, 1035-1045.

Rotszetejn H., Trznadel-Budzko E. and Jesionek-Kupnicka D. (2006). Langerhans cells in vulvar lichen sclerosus and vulvar squamous cell carcinoma. Arch. Immunol. Ther. Exp. 54, 363-366.

Rotszetejn H., Trznadel-Budzko E. and Jesionek-Kupnicka D. (2007). Do Langerhans cells play a role in vulvar epithelium resistance to squamous cell carcinoma. Arch. Immunol. Ther. Exp. 55,127-130.

Rutledge F.N., Mitchell M.F., Munsell M.F., Atkinson E.N., Bass S, McGuffee V. and Silva E. (1991). Prognostic indicators for invasive carcinoma of the vulva. Gynecol. Oncol. 42, 239-44.

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.

Schon M.P. and Ludwig R.J. (2005). Lymphocyte trafficking to inflamed skin - molecular mechanisms and implications for therapeutic target molecules. Expert. Opin. Ther. Targets. 9, 225-243.

Scurry J. (1999). Does lichen sclerosus play a central role in the pathogenesis of human papillomavirus negative vulvar squamous cell carcinoma? The itch-scratch-lichen sclerosus hypothesis. Int. J. Gynecol. Cancer 9, 89-97.

Scurry J.P. and Vanin K. (1997). Vulvar squamous cell carcinoma and lichen sclerosus. Austr. J. Dermatol. 38 (suppl 1). S20-25.

Steinman R.M. (1991). The dendritic cell system and its role in immunogenicity. Annu. Rev. Immunol. 9, 271-296.

Takeuchi Y., Tohnai I., Kaneda T. and Nagura H. (1988). Immunohistochemical analysis of cells in mucosal lesions of oral lichen planus. J. Oral. Pathol. 17, 367-373.

Tay S.K., Jenkins D., Maddox P., Campion M. and Singer A. (1987). Subpopulations of Langerhans' cells in cervical neoplasia. Br. J.

Obstet. Gynaecol. 94, 10-15.

Vayyrynen M., Syrjanen K., Mantyjarvi R., Castren O. and Saarikoski S. (1984). Langerhans cells in human papillomavirus (HPV) lesions of the uterine cervix identified by the monoclonal antibody OKT-6. Int. J. Gynaecol. Obstet 22, 375-383.

Walker F., Adle-Biassette H., Madelenat P., Henin D. and Lehy T. (2005). Increased apoptosis in cervical intraepithelial neoplasia associated with HIV infection: implication of oncogenic human papillomavirus, caspases, and Lagerhans cells. Clin. Cancer. Res. 11, 2451-2458.

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