ORIGINAL ARTICLE

Arch. Immunol. Ther. Exp., 2006, **54**, 363–366
PL ISSN 0004-069X

DOI 10.1007/s00005-006-0039-6

Langerhans cells in vulvar lichen sclerosus and vulvar squamous cell carcinoma

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Received: 2006.02.27, **Accepted:** 2006.07.20, **Published online first:** 2006.10.06

Abstract

Introduction: Langerhans cells (LCs), specializing in antigen presentation, are a very important part of the skin immune system (SIS).
Materials and Methods: Skin biopsies from 22 women with vulvar lichen sclerosus (LS): 15 patients with early and 7 with the late stage of the disease, were evaluated. Five women with vulvar squamous cell carcinoma (SCC) were also examined. The control group consisted of 9 women who underwent plastic surgery of the vulvar region. Immunohistochemical staining was performed on formalin-fixed paraffin-embedded tissues samples using antihuman CD1a antibody (NCL-CD1a-235, Novocastra).
Results: Increased numbers of LC stainings were present in early LS, whereas decreased numbers of these cells were present in late LS and in SCC compared with the control group.
Conclusions: This study showed that dysregulation of the SIS may lead to suppression of LCs in the vulvar epithelium and may be one of the reasons for a higher tendency for carcinogenesis in the vulvar region.

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

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INTRODUCTION

Langerhans cells (LCs) play an important role in the skin immune system (SIS), where they constitute 2–4% of epithelial cells [10]. These cells are part of a wide system of dendritic cells specializing in antigen presentation. They can migrate to regional lymph nodes and present antigens to naive T cells [11, 13]. Activated T cells can migrate to the skin and release cytokines (interleukin 1 and 2, tumor necrosis factor (TNF)-α), resulting in inflammatory infiltrates and skin diseases [8, 21]. This is highly important in the pathogenesis of inflammatory skin diseases such as psoriasis and atopic dermatitis. T cells also migrate to the skin in such malignancies as T cell lymphomas. Cytokines, chemokines, and adhesion molecules play a significant role in the process of lymphocyte migration. At the same time, inhibiting the processes connected with the recruitment of lymphocytes to the skin may be useful in treatment [16].

Lichen sclerosus (LS) is a chronic inflammatory skin disease affecting mainly the vulvoperineal area. Its clinical symptoms, which include intractable burning itch, may be associated with dysuria, dyspareunia, and dryness of the skin. The pathogenesis of LS is not completely clear. Many authors indicate that the SIS is involved in the disease [1, 2]. LCs play a crucial role in allergic contact diseases, viral diseases, graft-versus-host diseases, and the elimination of neo-plastic cells [10]. Moreover, literature data suggest an association of LS with an increased risk of the vulvar epithelial malignancy [1, 3, 14]. The aim of this study was to evaluate the number of LCs in the epithelium in early and late lesions of vulvar LS and vulvar squamous cell carcinoma (SCC).

MATERIALS AND METHODS

We evaluated skin biopsies taken from 22 untreated women with vulvar LS (mean age: 55.1±11.7 years). The diagnosis was confirmed clinically by reddish and white areas with atrophy. The patients did not have extragen-

ital lesions. Histopathological examination showed 15 cases with early stage of the disease, characterized by little atrophy of the epithelium and hyperkeratosis, a narrow homogenization zone under the epithelium, and superficial inflammatory cells consisting mainly of lymphocytes. Seven cases were of the late stage of LS, with more atrophic epithelium, a wide homogenization zone, and small inflammatory cells more deeply situated. We also examined 5 cases of SCC of the vulva obtained from women who underwent vulvectomy (mean age: 65.1±11.5 years). At the beginning of our examination, all the women had untreated changes in the vulvar region. The biopsies showed carcinogenesis. The cases of SCC were of well and moderate histopathological grade, with characteristic foci of keratinization. Adjacent tissues were normal. The control group consisted of 9 normal vulvar skin tissues obtained from surgical specimens of women (mean age: 57.3±11.2 years) who underwent plastic surgery 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- to 49-kDa molecule expressed in association with beta 2 microglobulin. CD1a is expressed strongly by cortical thymocytes, LCs in the epidermis, dendritic cells in the dermis, and also by LCs of the 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% H_2O_2 in methanol for 30 min and incubated with primary anti-CD1a antibody (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 staining, serum mouse IgG1 (Serotec) as an isotype control antibody against anti-CD1a antibody was used.

Evaluation of LC

Immunohistochemical staining revealed the LCs exhibited characteristic processes. The number of LCs in each case was estimated as the number of cells calculated per 10 high-power fields (HPF) at 400xmagnification.

Statistical analysis

The examined groups and the control group were compared by performing one test, the Mann-Whitney U-test.

RESULTS

Immunohistochemical staining revealed that the LCs exhibited cytoplasmatic characteristic processes (Table 1). The mean number of LCs in the control tissue as 6.6/10 HPF (range: 1–12; Fig. 1 and 2). In early LS the mean number of LCs was 25.8/10 HPF (range: 15–51, $p<0.001$ compared with the control group; Fig. 3). In late LS the mean number of LCs was lower: 2.3/10 HPF (range: 1–4, $p<0.01$ compared with controls; Fig. 4). Surprisingly, the number of LCs in SCC was very low, their mean number being 1.0/10 HPF (range: 0–4, $p<0.01$ compared with controls; Fig. 5).

Table 1. The number of Langerhans cells in the epithelium in different lesions and the control group (mean ±SD)

Groups	N	Range	Mean	SD	p
Control	9	1–12	6.6	3.2	
Early LS	15	15–51	25.8	10.7	$p<0.001$
Late LS	7	1–4	2.3	1.4	$p<0.01$
SCC	5	0–4	1.0	1.7	$p<0.01$

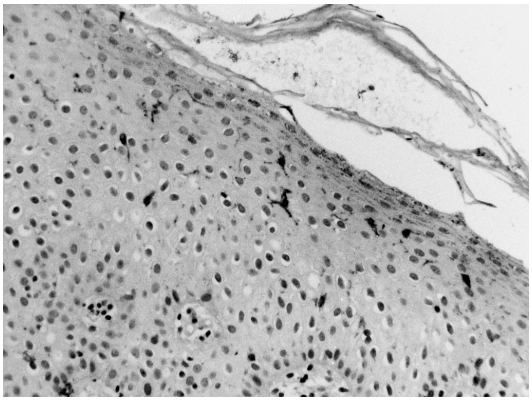

Fig. 1. The skin section of control tissue without pathological changes. Note a few LC with characteristic proccesses. Immunohistochemical staining with anti CD1a antibody, DAKO ENVISION System HRP DAB, hematoxylin counterstaining, ×400.

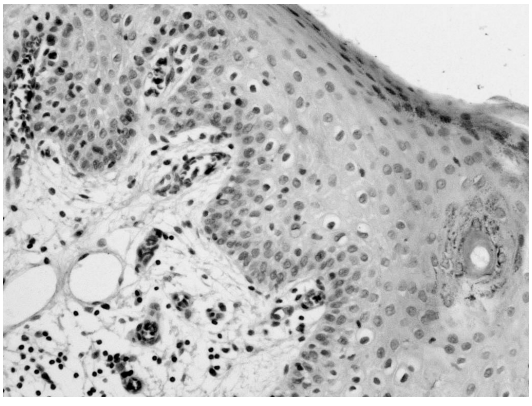

Fig. 2. Negative control with mouse serum IgG1 (Serotec) as isotype control antibody against ant-CD1a antibody, hematoxylin counterstaining, ×400.

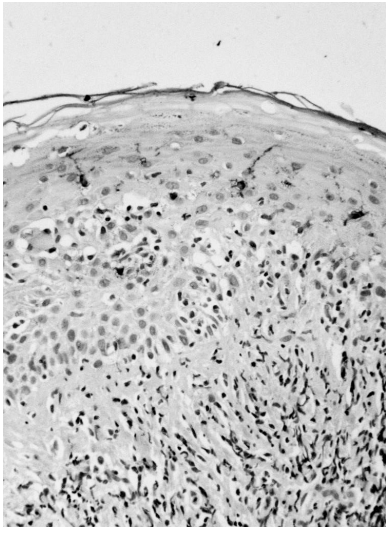

Fig. 3. The early lesions of lichen sclerosus – a large number of Langerhans cells. Immunohistochemical staining with anti CD1a antibody, DAKO ENVISION System HRP/DAB, hematoxylin counterstaining, ×400.

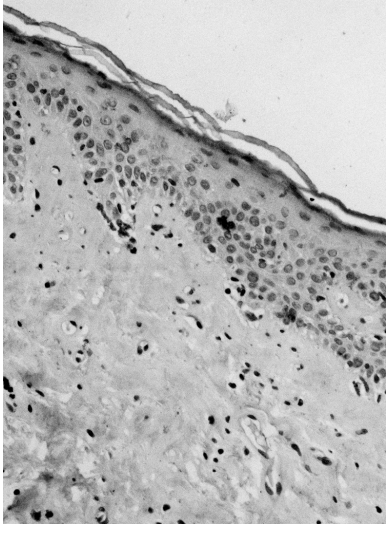

Fig. 4. The late lesions of lichen sclerosus – a small number of Langerhans cells. Immunohistochemical staining with anti CD1a antibody, DAKO ENVISION System HRP DAB, hematoxylin counterstaining, ×400.

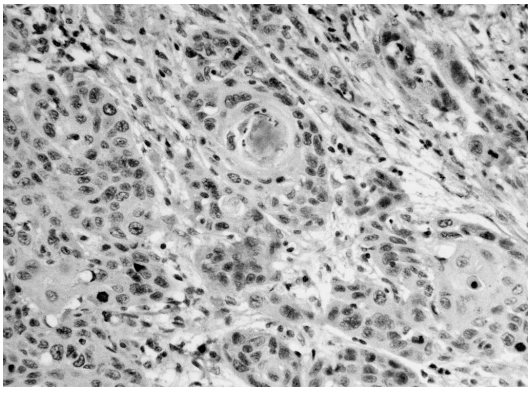

Fig. 5. The squamous cell carcinoma – the absence of Langerhans cells. Immunohistochemical staining with anti CD1a antibody, DAKO ENVISION System HRP DAB, hematoxylin counterstaining, ×400.

DISCUSSION

The precise pathomechanism of vulvar LS has not been elucidated. It seems clear that the recruitment of lymphocytes to the skin, like in other inflammation diseases [16], plays a significant role in the pathogenesis of LS.

The relationship between inflammation and cancer due to chronic infection has been identified in many solid tumors. Examples include *Helicobacter pylori*-induced gastritis in gastric cancer, inflammatory bowel disease (ulcerative colitis and Crohn's disease) in colorectal cancer, chronic viral hepatitis in liver cancer, and inflammation of the pancreas and oral lichen planus in squamous oral cancer [4, 5, 7, 9]. The most important factor in carcinogenesis is nitrative damage to nucleic acids, DNA and RNA, which occurs during inflammation through the generation of reactive nitrogen species, such as peroxynitrite, nitroxyl, and nitrogen dioxide. These cause the accumulation of potentially mutagenic DNA lesions, including 8-nitroguanine and 8-oxodG and the accumulation of p53 in damaged epithelium and inflammatory-related carcinoma [4, 9].

The recruitment of lymphocytes to the skin may be also connected with the recruitment of LCs to the skin. Our examination indicates that in early lesions there are more LCs than at the late stage of the untreated disease. At the same time, a decreased number of inflammatory infiltrates is known in the late stage disease, which may be directly connected with the suppression of LCs. This is proof that the activity of the SIS is lower in late lesions of LS. Carli [2] evaluated LCs in LS lesions. The author pointed out an increased number of LCs in vulvar LS. However, early and late stages of the disease were not described. The author applied clobetasol propionate and observed not only clinical and histological improvement, but also a decreased number of LCs in the epithelium [2]. Prignano et al. [13] reported that the *in vitro* activity and the number of LCs is higher in the epidermis after adding TNF-α and GM-CSF to sheets.

LCs make contact with keratinocytes by means of E-cadherin, molecules responsible for adhesion of epithelium cells [18]. Other authors pointed out that LCs and lymphocytes make contact through soluble and membrane molecules to make both types of cells more active [19]. Many authors reported larger numbers of LCs in the skin and mucosa in lichen planus changes. This was considered the result of a higher production of IL-1 by keratinocytes [15, 20]. The increased number of LCs was usually connected with their higher activity [12, 17]. Yamanaka et al. [21] suggested that there was a smaller number of LCs in the centers of the lesions of annular lichen planus with atrophic epidermis than outside the lesions. This correlated with the intensity of lymphocyte infiltrates. Our findings clearly show a large decrease in the LC counts in vulvar SCC. The extremely low level or absence of LCs may be directly connected with carcinogenesis. The number of LCs significantly decreased in the epidermis in Bowen's disease [6]. Ben-Hur et al. [1] reported that only two lymphoid markers were significantly different in LS compared to vulvar SSC. He observed a decreased number of T lymphocytes and an increased number of B lymphocytes in the infiltration [1], but he did not estimate LCs.

Our study has shown that the dysregulation of the SIS in LS may lead to the suppression of LCs in the vulvar epithelium at the late stage of the disease. At the same time, this may be one of the reasons for a higher tendency towards developing carcinogenesis in the vulvar region.

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