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Immunohistochemical Evidence of Skin Immune System Involvement in Vulvar Lichen sclerosus et atrophicus

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Abstract. Biopsies taken from vulvar lesions in 12 women affected by vulvar lichen sclerosus et atrophicus (LSA) have been processed for immunohistological study. Activated (HLA-Dr+) T cells, associated with CD1a+ accessory cells, were found in the dermis in all cases, with architectural patterns varying in relation to the histological phase (early, well developed, old) of the lesion. Interestingly, the number of epidermal CD1a+ Langerhans cells (LCs) was increased in all cases, without any correlation with the amount of the dermal infiltrate and with the histological phase of the lesions. In fact, also in old lesions the number of epidermal CD1a+ LCs was increased, and the sparse dermal lymphoid cells showed a persistent HLA-Dr antigen expression. These data, indicating the persistent activation of epidermal antigen-presenting cells and lymphoid cells in all the evolutive phases of vulvar LSA, suggest a possible involvement of the skin immune system in the pathogenesis of LSA.

Lichen sclerosus et atrophicus (LSA) is an uncommon chronic skin disease; it may affect both sexes but mainly occurs in prepuberal and postmenopausal women, showing a striking predilection for genital areas. The aetiology and pathogenesis of LSA are still unknown. A genetic predisposition has been reported in some patients [1, 2]. In addition, in women with vulvar LSA, low serum levels of dehydrotestosterone and androstenedione have been found [3]; reduced androgen-dependent 5α-reductase activity in perineal skin of these patients has thus been hypothesized, in agreement with the relatively good efficacy of local testosterone application and with the frequent clinical observation of puberal spontaneous improvement in affected children [3, 4]. On the other hand, several studies have shown a significant association of LSA with auto-immune diseases [5, 6], and the incidence of organ- and non-organ-specific serum auto-antibodies appears significantly increased in patients with LSA [5, 7].

Despite these data, the role of the immune system in LSA has not been completely clarified as yet. We analysed the immunohistological features of the cutaneous infiltrate in women with vulvar LSA, with the aim of evaluating the possible involvement of the skin immune system in the pathogenesis of vulvar LSA.

Materials and Methods

Patients

Twelve women with clinically typical, untreated vulvar LSA were analysed. The clinical diagnosis was histologically confirmed in all patients. The age ranged from 6 to 78 years (median = 52.5). Pruritus, soreness and dyspareunia were variably present in all cases. At the time of biopsy, the disease had been present for < 12 months in 2 cases (No. 4, 6); in the others, it had been present for longer periods. No patient had diabetes mellitus. The biopsies were performed on lesional vulvar skin, choosing similar clinical lesions, i.e. ivory-white hyperkeratotic, slightly atrophic lesions.

Immunohistochemistry

Skin biopsies were snap frozen and stored at –70 °C. Frozen sections (6 μm thick) were air dried, fixed in acetone for 5 min and stained by the avidin-biotin-peroxidase complex method described in a previous study [8]. The monoclonal antibodies used in this study and their

Table 1. Monoclonal antibodies used

Monoclonal antibody	CD code	Specificity	Source
OKT3	CD3	T cells	ODS
OKT11	CD2	E rosette receptor	ODS
OKT4	CD4	helper/inducer T cells	
OKT8	CD8	suppressor/cytotoxic T cells	
OKT6	CD1a	Langerhans cells	
Leu 14	CD22	B cells	BD
HLA-Dr		B cells, activated T cells, monocytes, Langerhans cells	
DRC-1		dendritic reticulum cells	BD
Anti-C3br	CD35	C3b receptor	
Leu M5	CD11c	monocyte-macrophage	BD
OKM5	CD36	monocyte-macrophage	ODS

ODS = Ortho diagnostic system; BD = Becton & Dickinson.

specificities [9] are listed in table 1. For quantitative analysis, the stained cells were counted in three contiguous microscopic fields in the dermis and in the epidermis (comprising basement membrane) at ×250. The results in the dermis were expressed semiquantitatively as the percentage of stained cells on a total of 100 cells; those in the epidermis were expressed as the number of stained cells overlying 100 basal cells. Frozen sections from 4 normal vulvar biopsies have been processed to evaluate the normal range of epidermal CD1a + dendritic cells. Only cells whose nucleus was contained in the plane of the section were considered. The lesions were classified as 'early', 'well developed' or 'old', according to the classic histological criteria [10]. An early histological pattern – i.e. infiltrate located in the upper dermis, close to the epidermis – was found in cases No. 1–3. Well-developed lesions – i.e. a conspicuous band-like lymphoid infiltrate located in the mid dermis, separated from the epidermis by prominent oedema – were observed in cases No. 4–9. The remaining cases (No. 10–12) showed a very scarce infiltrate, lymphoid cells being scattered in the upper and mid dermis, intermingled with swollen collagen fibres; this histological pattern has been categorized as old.

Results

The immunohistochemical findings in the dermis and in the epidermis are separately summarized in tables 2 and 3, respectively.

In the Dermis

In all cases, the majority of infiltrating cells expressed CD2 and CD3 antigens (fig. 1). The CD4/CD8 ratio varied from 2:1 to 1:1, without any difference among early, well-developed and old lesions. CD1a+ dendritic cells were constantly associated with T cell areas (fig. 2).

Fig. 1. The majority of dermal infiltrating cells express CD3 antigen. Immunoperoxidase. ×100.

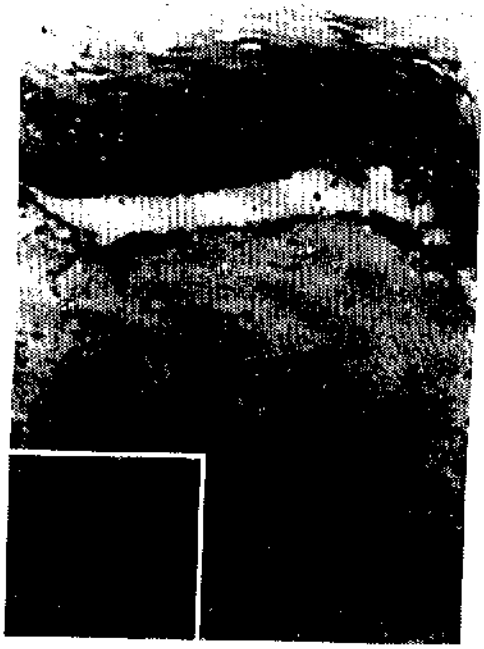

Fig. 2. Numerous CD1a+ dendritic cells are present in the epidermis. In the dermis, CD1a+ dendritic cells (arrowheads) are associated with infiltrating cells (case No. 4; well-developed lesion). Immunoperoxidase. ×100. **Inset:** Dendritic shape of dermal CD1a + cells. ×630.

Fig. 3. In the superficial dermis, close to the epidermis, a conspicuous number of CD11c-positive cells is found. Immunoperoxidase. ×100. **Inset:** Dendritic shape of dermal CD11c− cells. ×630.

Fig. 4. In the epidermis, HLA-Dr + dendritic cells are observed; conversely, no HLA-Dr staining of keratinocytes is found; in the dermis, the majority of infiltrating cells showed an activated phenotype, expressing the HLA-Dr antigen (case No. 2, early lesion). Immunoperoxidase. ×160.

Table 2. Dermal lymphoid infiltrate

Patient No.	Age years	CD3	CD4	CD8	CD1a+	CD11c	CD22	CD35	HLA-Dr
1	78	+−	+	+	+	+	−	−	+++
2	64	+	+++	++	+	++	−		+++
3	51	−+	++	+	−	++	−	−	+++
4	6	−++	++	−−	++	++	+	+	−−−
5	26	−+	++	−−	++	+−+	−	−	+++
6	30	−++	+++	−+	+	−+	+	+	++
7	60	−++	++	+	+	+	−	−	+++
8	57	−++	++	+	+	++	−	−	+++
9	54	−+	++	+	+	++	−	−	+
10	49	++	++	+	−	++	−		+++
11	69	+++	+−+	+	++	++		−	+++
12	42	+++	+	+	+	+	−	−	+

− = 0; − = < 10%; + − = 20–50% −++ = > 50%. Early lesion = cases No. 1–3; well-developed lesion = cases No. 4–9; old lesion = cases No. 10–12.

The number of CD1a+ cells varied in relation to the amount of dermal lymphoid T cells. In 2 cases only (No. 4, 6), in which the infiltrate showed an architectural pattern typical of well-developed lesions, were CD22+ lymphoid cells found, clustered with dendritic DRC-1+, CD35+ accessory cells, configurating a B follicle-like structure. Both in the superficial dermis and in the basal layer of the epidermis, CD11c+ cells were constantly recognized (fig. 3). Concerning the CD36 staining (performed in 5 cases only), we regularly observed perivascular CD36+ dendritic cells in the whole dermis.

The majority of infiltrating cells showed an activated phenotype, expressing the HLA-Dr antigen independently of the histological phase of the lesion (fig. 4).

In the Epidermis

In all cases, numerous CD1a+ dendritic cells were constantly found. Their number ranged from 25 to 30 cells overlying 100 epidermal basal cells (table 3), whereas the number of CD1a+ dendritic cells in the epithelium of clinically and histologically normal vulvar biopsies (n=4) ranged from 16 to 18 per 100 basal cells.

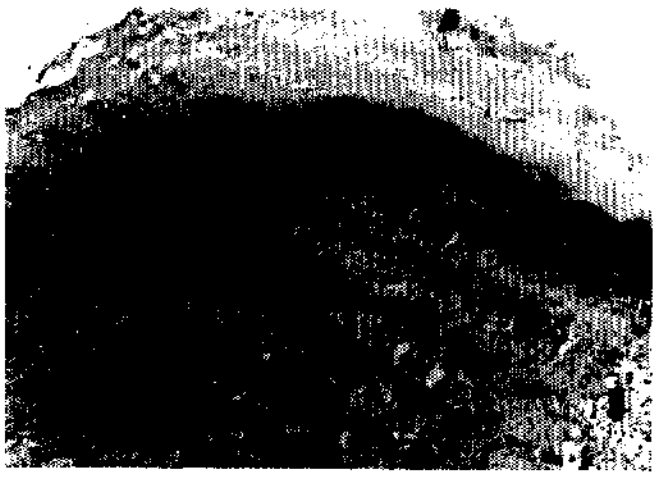

Fig. 5. In the early phase of a vulvar LSA lesion, numerous CD1a-positive dendritic cells are observed in the epidermis (case No. 1). Immunoperoxidase. ×100.

Fig. 6. In the old histological phase of a vulvar LSA lesion, numerous CD1a-positive dendritic cells are observed in the epidermis (case No. 11). Immunoperoxidase. ×160.

Table 3. Number of epidermal dendritic CD1a+ cells overlying 100 basal cells

Cases	1	2	3	4	5	6	7	8	9	10	11	12
CD1a+	27	28	26	30	25	29	27	25	26	28	25	28

Early lesion = cases No. 1–3; well-developed lesion – cases No. 4–9; old lesion = cases No. 10–12. Range in normal vulvar biopsies (n = 4): 16–18 CD1a+/100 basal cells.

This finding of numerous CD1a+ dendritic cells has been observed in all patients, independently of age, histological pattern and amount of the dermal infiltrate of the lesion (fig. 2, 5, 6). The CD1a + dendritic epidermal cells were located in the basal and suprabasal layers. Sparse CD2+/CD3+ (CD4+/ , CD8+/–) cells were frequently observed in the epidermis.

HLA-Dr + dendritic cells were regularly evidenced in the epidermis; conversely, no HLA-Dr staining of keratinocytes was found (fig. 4).

Virtually no CD36+ dendritic cells were found, while a keratinocyte suprabasal staining was observed in 3 of 5 cases in which such monoclonal antibodies have been used.

Discussion

The actiopathogenesis of LSA is unknown. Different pathogenetic hypotheses have been suggested, i.e. focussing on hormonal [3], enzymatic [11, 12] or infectious processes [13]. Familial cases have been reported [1, 2], and genetic aspects have also been stressed concerning the relationship between LSA and HLA type [14, 15]. According to the above-mentioned data, LSA is probably a multifactorial disease [7, 16].

The immune system seems strongly involved as well. Several reports have indicated an association between LSA and auto-immune diseases [5, 7], and a significant percentage of women with vulvar LSA show serum autoantibodies [7]. Despite these data, little is known on the immunohistological features of vulvar LSA. Dickie et al. [17] reported immunoglobulin and complement deposition in the basement membrane zone in some vulvar LSA patients.

In our study, a well-defined immunohistological profile has been found: an activated (HLA-Dr+) T lymphocyte infiltrate has been observed in all patients, associated with dermal CD1a+ dendritic cells, thus creating a classic T zone micro-environment; in the atrophic epidermis, the number of CD1a+ dendritic cells – presumably Langerhans cells (LCs) – was constantly increased as compared to the normal value of epidermal LCs in the vulvar epithelium, according to our data and those of the literature [18]. Interestingly, this finding did not appear related to the amount of the dermal lymphoid infiltrate, having been also found in old lesions with scarce infiltrate and evident fibrosis. This finding could be explained as a sign of persistent activation of the afferent limb of the skin immune system. In this view, one can speculate that the numerous

CD11c+ cells found in the infiltrate, mainly located in the papillary dermis close to the epidermis, could represent a precursor pool of cells which are going to acquire the features of fully differentiated LCs within the epidermis [19]. The activation of LCs – supported by the quite large proportion of HLA-Dr+ dendritic cells in the epidermis – might be responsible, via interleukin-1 production, for the induction of a T-cell-mediated response [20] and for the subsequent lymphokine release [21]. From this point of view, the recent report of a reduction of the density of epidermal LCs in mice following the topical application of testosterone dipropionate [22] might explain the well-known beneficial effect of local testosterone in the treatment of vulvar LSA.

The follicle-like structures (CD22+ B cells clustered with DRC-1+ dendritic cells), found only in 2 cases showing a heavy T cell infiltrate, mainly CD4+, may be interpreted as a reactive process presumably due to the release of cytokines by infiltrating T cells. In fact, this finding has also been shown in other conditions characterized by a heavy cutaneous band-like infiltration of CD4+ cells [23]. In conclusion, the skin immune system seems actively involved in the pathogenesis of the vulvar LSA; the persistent activation of the afferent limb of the skin immune system, as suggested by the persistently increased number of epidermal LCs in all the evolutionary phases of the disease, could explain the progressive and not self-limited course of LSA. In this view, the sclerofibrotic evolution of vulvar LSA lesions cannot be merely considered as a sign of extinguishment of disease activity.

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