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Differences in Extracellular Matrix Proteins, Epidermal Growth and Differentiation in Discoid Lupus Erythematosus, Lichen Planus and the Overlap Syndrome

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Lichen planus (LP) and discoid lupus erythematosus (DLE) are separate disease entities. Nevertheless, patients with a so-called "overlap syndrome" have been described occasionally. The aim of the present study was to establish whether the LE/LP overlap syndrome, based on clinical and routine histological features, could be delineated from DLE or LP using immunohistochemical techniques. Formalin-fixated, paraffin-embedded skin biopsies of patients with DLE, LP and the overlap syndrome were compared regarding immunohistochemical markers for epidermal growth and differentiation and extracellular matrix components. With the markers for extracellular matrix proteins, it was possible to delineate the overlap syndrome from LP. This was not possible for the overlap syndrome and DLE. These findings might indicate that the LE/LP overlap syndrome could be considered as LP-like DLE rather than as a distinct disease entity. ***Key words: immunohistochemistry; tenascin; laminin; heparan sulphate.***

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Lichen planus (LP) and discoid lupus erythematosus (DLE) are separate disease entities. Nevertheless, patients with features of both diseases, the so-called lupus erythematosus/lichen planus (LE/LP) overlap syndrome, have been described (1–5). No communis opinio exists as to whether the overlap syndrome is a distinct disease or part of a spectrum. Various investigators have studied the LE/LP overlap syndrome with regard to the clinical picture of the lesions (1, 2), histopathology and immunofluorescence (2, 6, 7), serological differences or genetic background (8).

LP and DLE are histologically distinct diseases but share some features as well. Both are immunologically mediated interface dermatoses, are inflammatory and show slight hyperproliferation (9–11). The inflammatory infiltrate expresses generally the same amounts of the lymphocyte activation marker HLA-DR (6). In addition, using immunohistochemical markers for differentiation, it was shown that in DLE premature terminal differentiation is combined with normal early differentiation (10). The extent to which these features are deviant in patients with LP or the LE/LP overlap syndrome is unknown. Histological investigations, using H&E sections and immunofluorescence of the LE/LP overlap syndrome, have been carried out in order to delineate the syndrome from DLE and LP, with the combination of the two techniques giving the most reliable results to distinguish between DLE and LP (7). Nevertheless, patients with the LE/LP overlap syndrome could not be clearly delineated using these parameters (2, 3, 5, 7).

The aim of the present study was to establish whether the LE/LP overlap syndrome, based on clinical and routine histological features, could be delineated from DLE or LP using immunohistochemical techniques. To address this question, we chose a panel of antibodies, focusing on epidermal growth (Mib-1) and differentiation (keratin 10, keratin 13/16, involucrin), together with antibodies staining extracellular matrix proteins (laminin, heparan sulphate and tenascin) (11–18). To the best of our knowledge, there is no information on the expression of extracellular matrix proteins in DLE, LP or the LE/LP overlap syndrome.

MATERIAL AND METHODS

Patients

In total, 32 patients were included in this study: 16 patients with clinical and histological features of both LP and DLE, 8 patients with the classic features of DLE (including positive lupus band test), and 8 patients with the classic features of LP (lupus band test negative). Epidemiological parameters (age, duration of illness, previous therapies) were recorded. The clinical picture was examined carefully. Presence of itching/painful sensations, localisation of the lesions, degree of atrophy, hyperpigmentation, and sensitivity to sunlight were recorded. The patients had not used topical or systemic treatment for at least 4 weeks before the biopsy was taken. They were considered to have the LE/LP overlap syndrome when at the same time lesions were present with features of both diseases, and/or when histological features of both diseases were present in the same skin specimen. Histological features compatible with DLE were: follicular plugging, hyperkeratosis, perivascular and perifollicular patchy infiltrates, vacuolar degeneration of the basal layer, atrophy of the epidermis, and positive lupus band test. The diagnosis of LP was based on the presence of a band-like infiltrate, saw-tooth formation, Civatte bodies, and negative lupus band test.

Immunohistochemistry

Biopsies were fixed in 4% paraformaldehyde and paraffin-embedded, and 6-μm sections were adhered to slides coated with 3-aminopropyltriethoxy-silane (Sigma, St Louis, MO, USA). Sections were deparaffinized through xylene and rehydrated through a graded series of ethanols. Normal controls and blancs were included. The following antisera were used for stainings on paraffin sections. To assess epidermal proliferation monoclonal antibodies were used against a nuclear antigen present in the cycling cell, Ki-67 (Mib-1, Immunotech S.A., France) and against keratin 16 and 13 (Ks8.12, Sigma, St Louis, MO, USA). Keratin 16 is present in hyperproliferative epidermis, while keratin 13 has not been found in adult human skin. To characterise epidermal differentiation the monoclonal antibody against keratin 10 (ICN Biomedicals, Zoetermeer, The Netherlands) was used and a monoclonal antibody against involucrin (MON-150) (19). Dermal extracellular matrix components were visualised using the antibody anti-tenascin (Telios, San Diego, USA), directed against the dermal glycoprotein tenascin. Immunostaining of heparan sulphate and laminin was performed using JM-403 against the heparan sulphate side-chain of heparan sulphate proteoglycan (a kind gift from J. van den Born, Dept. of Nephrology, University Hospital Nijmegen)

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and a polyclonal rabbit antiserum against laminin (ICN, Biomedicals, Zoetermeer, The Netherlands).

An indirect peroxidase technique was used for stainings with Ks8.12, anti-cytokeratin 10, anti-heparan sulphate and Mib-1. Briefly, the slides were pre-treated for 15 min in 0.1% trypsin/0.1% $CaCl_2$, pH 7.8, by 37°C for cytokeratin 10 and heparan sulphate. For Mib-1 two 5-min pretreatments with 10 mM citrate buffer, using a microwave oven at 450 Watt, were performed. After preincubations with 20% normal rabbit serum the slides were incubated for 60 min with the monoclonal antibodies, and after washing with phosphate-buffered saline (PBS) they were incubated with rabbit-anti-mouse immunoglobulin conjugated with peroxidase (RAMPO) 1:100 for 30 min. A solution of 3-amino-9-ethylcarbazole (AEC) in sodium-acetate buffer, pH 4.9, containing 0.01% H_2O_2, was added for 15 min after preincubation with sodium-acetate buffer, pH 4.9.

Immunostainings for tenascin and laminin were performed using a labelled avidin-biotin peroxidase technique. The slides for anti-tenascin were pretreated with 10 mM citrate-buffer, pH 6.0, for 30 min and 0.1% pronase 20 min for anti-laminin. After preincubation with 20% normal goat serum the slides were incubated with goat-anti-rabbit-biotinylated IgG (Vector Lab. Inc., Burlingame, USA) 1:100 for 30 min. After two washes with PBS, incubation with the avidin-biotin-peroxidase complex (Vector Lab. Inc., Burlingame, USA) 1:100 was performed. Then the slides were incubated with sodium-acetate buffer, pH 4.9, and finally stained in the AEC solution. All slides were counterstained with Mayer's haematoxylin (Sigma, St Louis, MO, USA) and mounted in glycerol-gelatine.

Scores

Mib-1-stained nuclei were counted per mm length of the section. Staining with the antibodies against keratin 13/16 and keratin 10 was assessed using the following scale: 0=no staining, 1=sporadic staining, 2=minimal staining, 3=moderate staining, 4=moderate-pronounced staining, 5=pronounced staining, 6=whole epidermis stained. MON-150 staining was expressed as percentage of stained epidermal cell layers per total number of cell layers. Staining for heparan sulphate and laminin was expressed as 0=no staining present, 1=discontinuous staining, 2=continuous staining. Tenascin was assessed as follows: 0=no staining, 1=discontinuous staining just beneath the epidermis, 2=continuous staining just beneath the epidermis, 3=discontinuous staining just beneath the epidermis and in the lower parts of the dermis, 4=continuous staining just beneath the epidermis and in the lower parts of the dermis.

Statistical analysis

Data are expressed as mean ± standard error of the mean. Statistical analysis was carried out using the Kruskal Wallis test. A p-value < 0.05 was supposed to be statistically significant.

RESULTS

Patients

The patient groups consisted of 8 patients with classic DLE (6 female, 2 male), 8 patients with LP (4 female, 4 male) and 16 patients with overlapping features (11 female, 5 male). Mean ages in years of these groups were 53.0 ± 6.4 (SEM), 49.3 ± 5.7, and 49.3 ± 3.8, respectively. The mean time the patients had suffered from their disease was 73.5 months ± 25.3 (SEM) in the DLE group, 25.1 ± 14.0 in the LP group, and 45.9 ± 16.8 in the LE/LP overlap group. Clinical features are illustrated in Table I.

Table I. *Clinical features of the three patient groups*

Clinical picture	DLE ($n=8$)	LP ($n=8$)	Overlap syndrome ($n=16$)
Location of the lesions:			
Face	7	–	11
Scalp	3	–	5
Extremities	–	8	11
Trunk	1	3	9
Mucosa	–	2	1
Morphology:			
Atrophy	8	1	13
Hyperpigmentation	–	3	3
Complaints:			
Itching	3	8	12
Pain	2	–	6
Provocation by sunlight	5	–	8
Localisation of biopsy:			
Face	6	–	2
Scalp	1	–	3
Extremities	–	8	7
Trunk	1	–	7

Immunohistochemistry

Results of the immunohistochemical investigations are shown in Figs. 1–3. In DLE and in the LE/LP overlap syndrome the numbers of Mib-1-positive cells were comparable (75.4 ± 31.6 (mean ± SEM), and 70.7 ± 9.9, resp.). In LP, however, the numbers of cycling cells were much lower (42.9 ± 9.1) but did not reach significance compared to biopsies from the LE/LP overlap syndrome patients.

Ks8.12 binding and keratin 10 were present in the suprabasal compartment in all three groups (DLE 3.6 ± 0.5, LP 3.6 ± 0.5, LE/LP overlap syndrome 2.7 ± 0.4 for Ks8.12, DLE 4.3 ± 0.2, LP 3.7 ± 0.2, and LE/LP overlap syndrome 3.6 ± 0.3 for keratin 10). No statistically significant differences between the three disease categories could be found. Involucrin expression was increased in all three groups, but no statistically significant differences could be found between DLE (67.1 ± 6.2), LP (65.4 ± 4.3) or the LE/LP overlap syndrome (66.6 ± 4.2).

Heparan sulphate and laminin, mainly present in the basal lamina, showed clear differences in the three disease categories. Heparan sulphate staining was decreased in LP (0.7 ± 0.2) compared to DLE (1.6 ± 0.2) and the LE/LP overlap syndrome (1.6 ± 0.01). The staining pattern was discontinuous and patchy in LP. Delineation from the LE/LP overlap syndrome was possible ($p=0.006$). In addition, the difference between LP and DLE was statistically significant ($p=0.01$). Laminin staining was clearly decreased in LP (0.9 ± 0.2) compared to the LE/LP overlap syndrome (1.5 ± 0.2, $p=0.02$). In DLE, laminin staining was comparable to the LE/LP overlap syndrome (1.2 ± 0.2).

The extracellular matrix glycoprotein tenascin was increased in all three disease categories. Tenascin staining was most notably increased in LP. Values were 2.7 ± 0.4 in DLE, 3.1 ± 0.3 in LP, and 1.9 ± 0.3 in the overlap syndrome. The difference in staining pattern between LP and the LE/LP overlap syndrome was statistically significant ($p=0.03$).

DISCUSSION

The first description of 4 patients with the LE/LP overlap syndrome was published by Copeman et al. in 1970 (1).

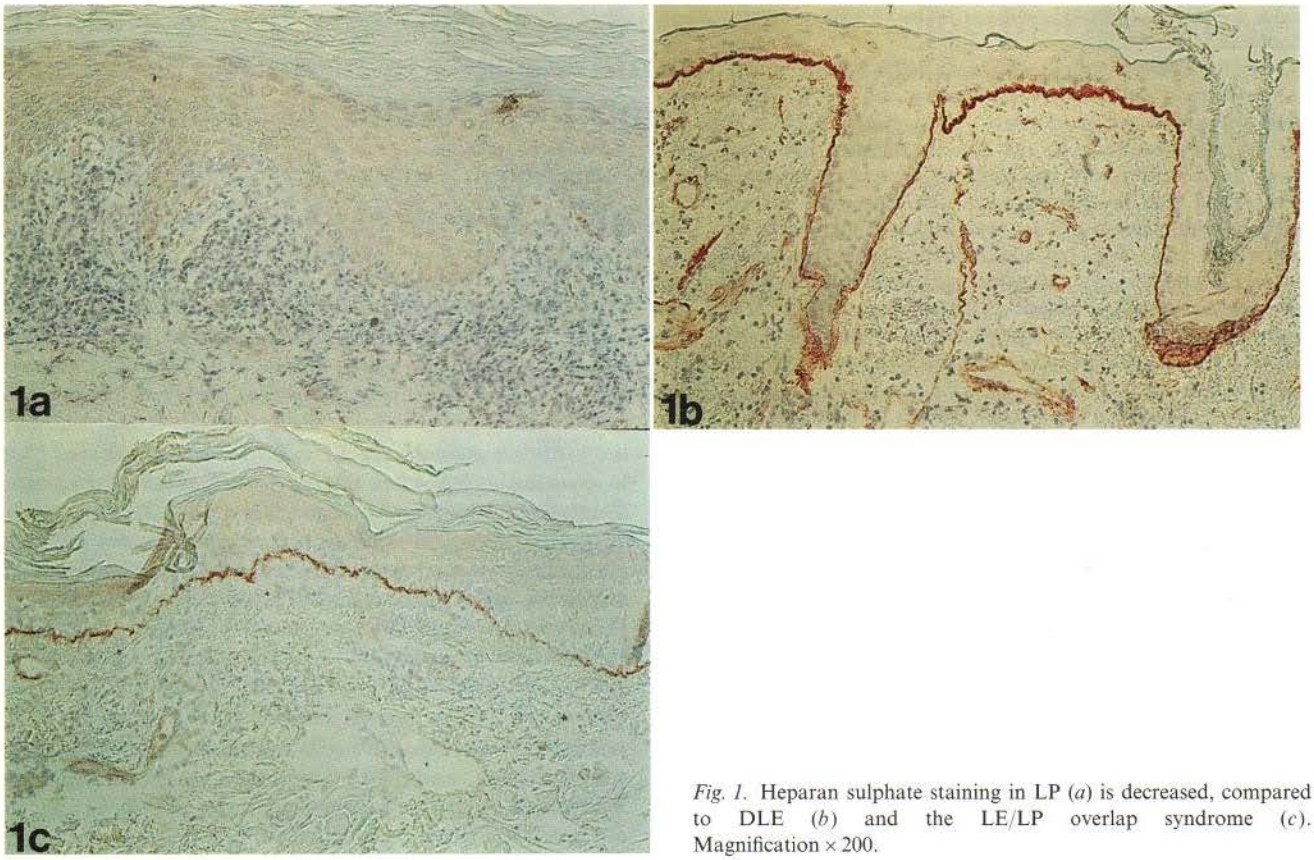

Fig. 1. Heparan sulphate staining in LP (*a*) is decreased, compared to DLE (*b*) and the LE/LP overlap syndrome (*c*). Magnification × 200.

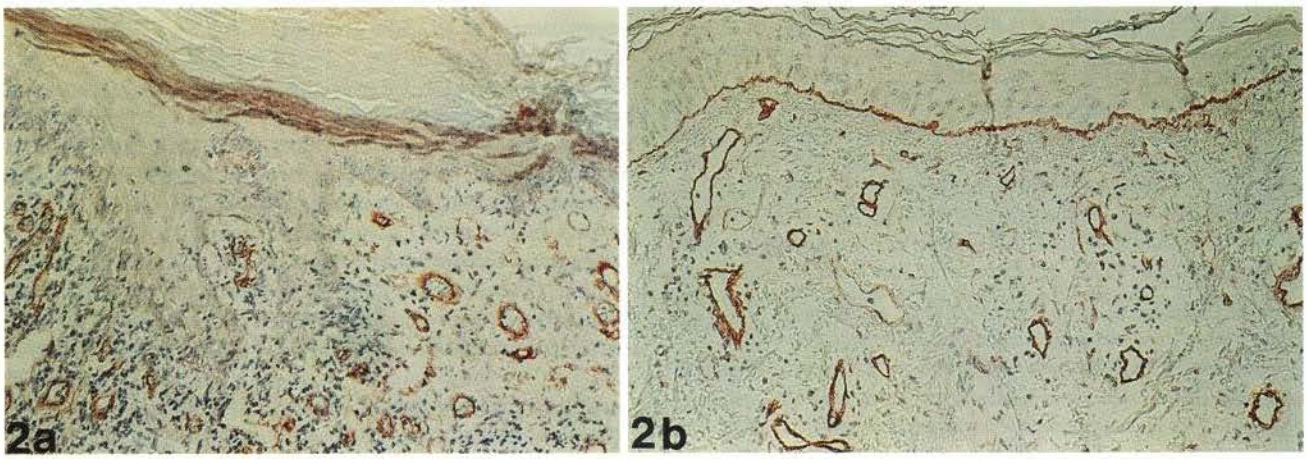

Fig. 2. Laminin staining in LP (*a*), decreased compared to the LE/LP overlap syndrome (*b*). Magnification × 200.

Subsequent reports postulated additional clinical and laboratory parameters that could differentiate between LP, DLE and the LE/LP overlap syndrome, such as clinically distinct types of skin lesions and a lupus-specific antigen in the sera of patients with LP, which should also be present in patients with the overlap syndrome (3). Differences in IL-2 receptors on T-lymphocytes were found (6), a speckled pattern of antinuclear antibodies was described (5), and differences in HLA-types were seen between DLE, LP and the overlap syndrome; a similar immune response in patients with different genotypes could lead to differences in clinical presentation of the disease (20). Otherwise, the hypothesis was postulated that these diseases form a spectrum and that there are no clear criteria to distinguish between them (2). The LE/LP overlap syndrome does not seem infrequent and is difficult to treat, although successful treatment with acitretin has been described (2, 5, 21).

In this study, skin biopsies from patients with the classic forms of DLE or LP were compared to patients with clinical and/or histological characteristics of both diseases. Staining of extracellular matrix components in the dermo-epidermal zone and the upper dermis showed clear differences between the three disease categories. In LP, a discontinuous staining pattern of heparan sulphate was found; this is in contrast to the continuous staining pattern of normal skin (22). The used antibody, JM403, is directed against low-sulphated domains

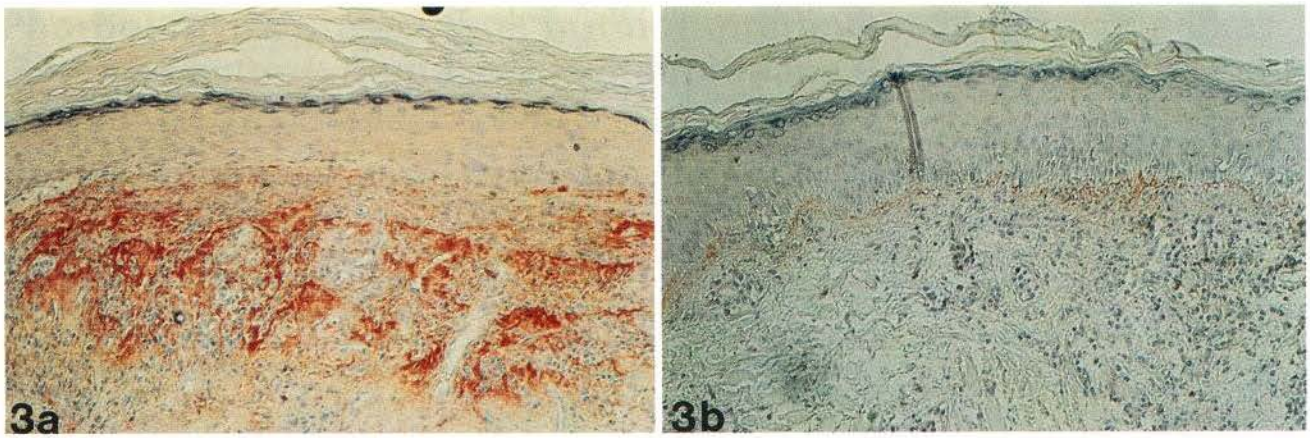

Fig. 3. Tenascin staining in LP (*a*), clearly increased compared to the LE/LP overlap syndrome (*b*). Magnification × 200.

of heparan sulphate (23). Heparan sulphate proteoglycans are part of the basal lamina of various tissues. They provide mechanical stability, support cell-membrane-basal lamina interactions, bind growth factors and influence charge-dependent transport of molecules (18). In DLE and the LE/LP overlap syndrome, heparan sulphate is also distributed discontinuously, as indicated by the antibody JM403. From a previous study, it is known that in DLE, the core protein of heparan sulphate proteoglycan is unaffected and continuously distributed along the basal lamina. (10) The decreased staining of heparan sulphate side-chains might represent a decreased presence and/or an impaired function as a reservoir for growth factors or as a substrate for cell-membrane-basal lamina interactions. This might form the biological counterpart to impaired wound healing or atrophy of the epidermis in these three disease categories.

Staining with a general antibody against laminin showed a decreased expression of laminin in LP. Laminin is one of the most important constituents of the basal lamina, providing resilience, adherence of keratinocytes to collagen IV in the basal lamina, and forms an important dermo-epidermal bridge (24). In the LE/LP overlap syndrome, laminin staining was more pronounced, compared to LP or DLE. The difference between the overlap syndrome and LP was statistically significant. Decreased staining of laminin, as seen in LP, might represent basal lamina damage and consecutively impaired basal lamina function, giving lymphocytes the opportunity to traffic to the epidermis.

The staining pattern of tenascin was increased profoundly in all three diseases, but it proved to be most prominent in LP. Delineation of the LE/LP overlap syndrome from LP was possible using this marker. The staining pattern of tenascin has been studied in various skin diseases. It was postulated that tenascin expression is increased in hyperproliferative skin diseases such as psoriasis, skin tumours and wound healing (25, 26). In addition, a correlation was seen between inflammation and the expression of tenascin (27). The exact function of tenascin is not yet known, and it is not known whether tenascin is induced and/or regulated primarily by fibroblasts or influenced by keratinocytes or inflammatory cells. In LP, a prominent damage of the basal lamina is observed, which is indicated by decreased heparan sulphate and laminin staining. It is intriguing that this skin disorder, which shows the most pronounced loss of basal lamina components, shows the highest tenascin expression. This might indicate that the interaction of dermal and epidermal components primarily induces tenascin.

Using the parameters for epidermal growth and differentiation, no clear distinction could be made between the LE/LP overlap syndrome and DLE or LP.

In conclusion, pronounced changes in the epidermal compartment as well as in the dermo-epidermal interface, as compared to normal skin, are seen in DLE, LP and the LE/LP overlap syndrome. Delineation of the LE/LP overlap syndrome from LP is possible using markers for extracellular matrix components in the dermo-epidermal zone but is not possible using markers for epidermal growth and differentiation. Delineation of the LE/LP overlap syndrome from DLE was not possible. This indicates that the LE/LP overlap syndrome should be considered as LP-like DLE rather than as a distinct disease entity. Further specification of changes in extracellular matrix proteins in the dermo-epidermal interface can provide more insight into the maintenance and pathogenesis of these diseases.

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