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Expression of Lymphocyte Activation Markers in Benign Cutaneous T Cell Infiltrates. Discoid Lupus Erythematosus versus Lichen Ruber Planus

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The expression of lymphocyte activation markers (IL2 receptors, transferrin receptors and HLA-DR) was examined in cutaneous lymphoid infiltrates of 12 patients with lichen ruber planus (LP) and 10 individuals with discoid lupus erythematosus (DLE). The cell infiltrates in both conditions were generally of considerable size. The vast majority of the infiltrating cells were T cells. The reactivity of the anti-IL2 receptor antibody used was confined to lymphocytes. In patients with LP 26 ± 17% of the infiltrating cells were IL2 receptor positive, 20 ± 8% carried transferrin receptors and >90% HLA-DR. In patients with DLE <1% were IL2 receptor positive, <5% carried transferrin receptors and >90% were HLA-DR positive. These data indicate that IL2 receptor expression distinguishes the infiltrating T-lymphocytes in LP and DLE, although in both conditions the vast majority of the infiltrating cells were activated as revealed by their expression of HLA-DR.

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During the course of skin and connective tissue diseases lymphocytes frequently infiltrate tissues where they normally occur in relatively limited numbers (1). The mechanisms underlying such pathological lymphocyte infiltration are generally unknown. The present investigation is an attempt to distinguish pathological cutaneous lymphocyte infiltrates due to their expression of surface markers associated with activation to blast transformation. Cutaneous disorders characterized by lymphocyte infiltration include both malignant conditions, e.g. lymphoma, and benign dermatoses. Patients with lichen ruber planus (LP) and discoid lupus erythematosus (DLE) display massive lymphocyte infiltration at the dermoepidermal junction and subepidermal regions, respectively. In LP and DLE as well as in other cutaneous disorders with lymphocyte infiltration the vast majority of the infiltrating cells are T-lymphocytes (2–4). After stimulation by antigen or mitogen, T cells secrete IL2 and develop receptors for this growth factor (5). Interactions between IL2 and its receptor regulate the growth of T cells (6). IL2 receptor expression and expression of other activation markers may provide a means for distinguishing disease states and their intensity. Immunohistological analysis of skin biopsies has demonstrated expression of IL2 receptors in both benign and malignant disorders (7–9) and in HTLV positive and negative disorders (9). Among the benign disorders, infiltrating lymphocytes in patch tests, eczema and psoriasis display weak IL2 receptor expression whereas in LP infiltrating cells display a more pronounced IL2 receptor expression (9). Here we report that IL2 receptor expression in skin infiltrating T lymphocytes is markedly higher in LP than in DLE.

MATERIAL AND METHODS

Patients and controls

Punch biopsies were taken from diseased skin of 12 untreated patients with LP and 10 untreated cases with DLE. The age of

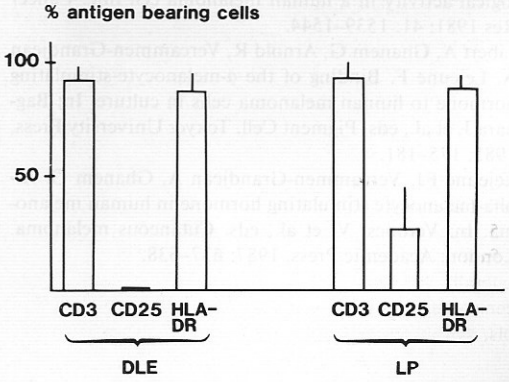

Fig. 1. Expression of lymphocyte activation antigens on infiltrating mononuclear cells in biopsies from patients with DLE and LP. Vertical bars represent standard deviation.

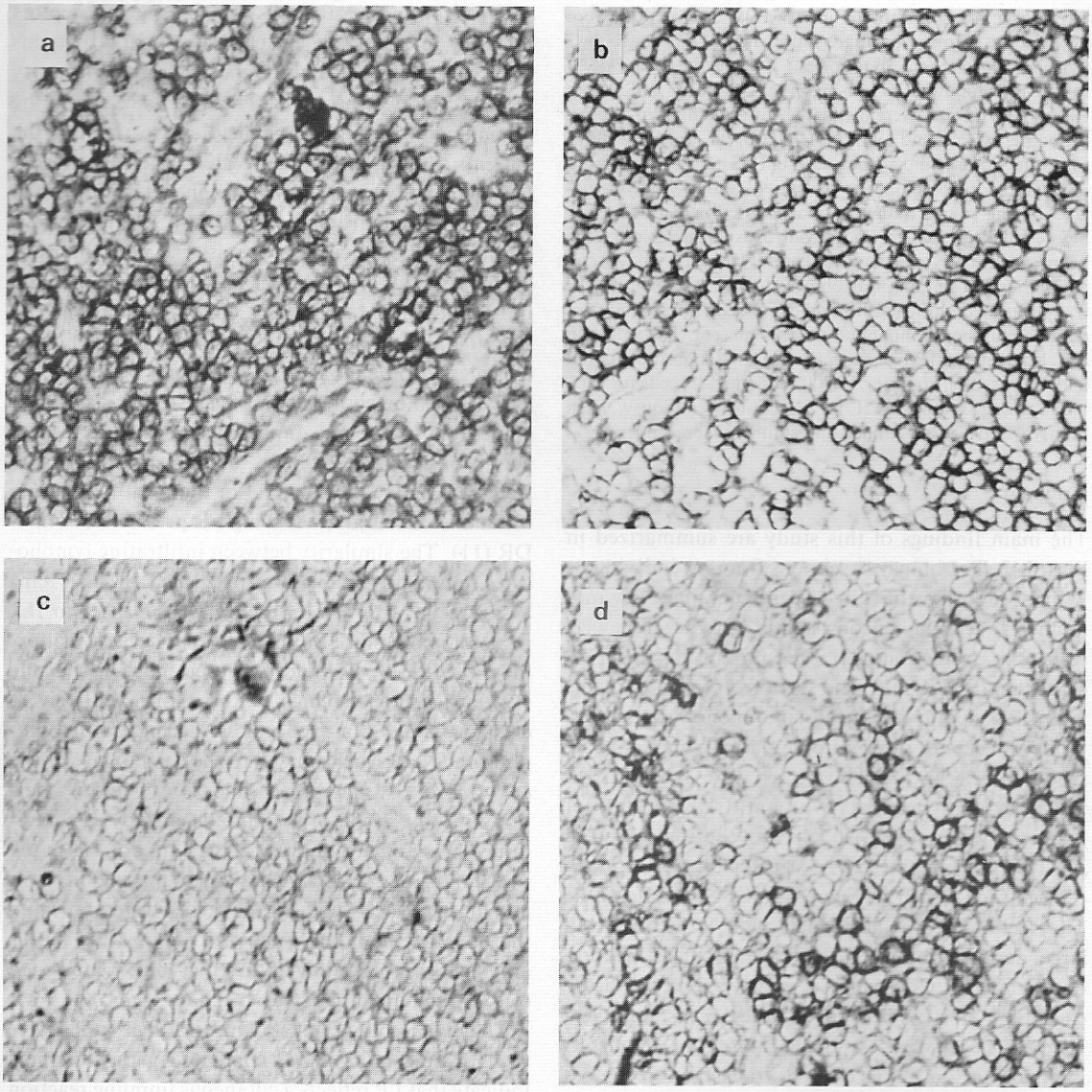

Fig. 2. CD3 expression (*a, b*) and IL-2 receptor expression (*c, d*) in infiltrating cells in biopsies from patients with DLE (*a, c*) and LP (*b, d*), respectively.

the LR patients ranged from 31 to 60 years, 5 males and 7 females. The age of the DLE patients ranged from 28 to 70 years, 5 males and 5 females. The duration of the disease varied from 1 to 6 months. The initial lesion of the DLE cases was generally provoked by sun exposure but the patients had not been sun-exposed during the month preceding biopsy. As controls, skin biopsies from 6 healthy individuals were applied.

Monoclonal antibodies

Monoclonal antibodies against CD25 (the IL2 receptor), CD3, CD4 and the transferrin receptor were obtained from Becton Dickinson (Mountain View, Ca). Monoclonal antibodies against CD8 antigen and HLA-DR were obtained from Ortho Diagnostic Systems Inc. (Raritan, NJ) and anti-B1 (36 kD) from Coulter Electronics (Luton, England).

Immunohistochemical staining

The biopsies were frozen in liquid nitrogen and frozen sections, 6 µm thick, were produced with a cryostat. After fixation in acetone for 10 min at 4°C the sections were rinsed in 9.3 mM phosphate buffered saline (0.14 M, $Na:K^+$ ratio 38:1) for 3×7 min at 20°C. The sections were then incubated for 30 min with monoclonal antibodies. Again the sections

were rinsed for 7 min three times in PBS. The sections were exposed to affinity purified biotinylated antimouse IgG (25 μg/ml, Vector Laboratories, Burlingame, Ca, USA) for 30 min followed by rinsing 7 min twice in PBS. After rinsing, the sections were incubated with a complex of biotinylated peroxidase and avidin DH (17 μg/ml, Vector Laboratories) for 45 min and again rinsed in PBS. The sections were incubated with peroxidase substrate (3-amino-9-ethylcarbazole) during 15 min and were finally rinsed in distilled water before mounting in glycerin. In control experiments when the primary antibody was omitted or replaced by anti-B cell antibodies, no staining was observed. The counting of cells was performed in the dermis and expressed as percentages of the total number of mononuclear cells present. The values in Fig. 1 and in the text represent mean ± standard deviation and are based on counting of 500 mononuclear cells for each monoclonal antibody in 1 to 3 sections per biopsy. Each biopsy was usually sectioned and stained several times.

RESULTS

The main findings of this study are summarized in Figs. 1–2. The biopsy specimens from six individuals without any signs of skin disease contained few mononuclear cells which consistently were negative with respect to expression of IL2 receptors, transferrin receptors and HLA-DR. The sections from individuals with LP generally displayed large mononuclear infiltrates adjacent to the dermo-epidermal junction. Virtually all cells in these infiltrates were T cells as revealed by their reactivity with anti-CD3 and lack of reactivity with anti-B1. 26 ± 17% of these T-cells were IL2 receptor positive, 20 ± 8% carried transferrin receptors (not shown) and > 90% HLA-DR. The sections from individuals with DLE displayed large dermal lymphocyte infiltrates consisting predominantly of CD4 antigen positive cells. With the exception of a small portion, these cells were IL2 receptor negative. Less than 5% of the cutaneous lymphocytes in DLE carried transferrin receptors (not shown) and > 90% were HLA-DR positive. There was no evidence that the monoclonal anti-IL2 receptor antibody used in the present study reacted to Langerhans' cells and macrophages as previously reported for anti-Tac (9). In parallel with the analysis of biopsy material, blood lymphocytes from individuals with LP were studied with respect to expression of IL2 receptors and other activation markers. Virtually no lymphocytes carrying IL2 receptors were found in the blood of three individuals.

DISCUSSION

Thus, the proportion of skin infiltrating lymphocytes which expressed IL2 receptors was "high" in LP whereas few infiltrating cells expressed such receptors in DLE. It is unlikely on clinical grounds that this difference reflected a difference in disease activity between the two disorders. The difference cannot be accounted for by differential medical treatment of the patients since these were untreated. The concept that IL2 receptor expression by T lymphocytes is linked to their activation from the resting to the replicative phase of the cell cycle (5, 6) raises several questions concerning the difference in IL2 receptor expression in LP and DLE, respectively. In both conditions virtually all cells displayed HLA-DR antigen, which is considered to be an activation antigen in T lymphocytes (10). Furthermore, previous studies have concluded that the local lymphocytes in various disease conditions characterized by mononuclear cell infiltrates are active participants in the local pathogenic mechanisms based on the high expression of HLA-DR (11). The similarity between infiltrating lymphocytes in LP and DLE with respect to HLA-DR is hard to reconcile with the difference with respect to IL2 receptor expression. In vitro findings suggest that expression of HLA-DR and IL2 receptors is dissociable (12) but such a dissociable expression is unlikely to exist in patients in vivo unless the infiltrating lymphocytes were synchronized to the same phase of the growth cycle. It may be possible to resolve this question by comparison of the amount of DNA as determined by cytometry in infiltrating lymphocytes in the two conditions. Recent studies comparing in vitro and in vivo activated lymphocytes have indicated that most of the in vitro activated Ia positive T cells are blasts whereas most of the in vivo activated such cells are small lymphocytes (13).

The high frequency of skin infiltrating lymphocytes with IL2 receptors in LP compared with DLE may indicate that LP reflects a cutaneous immune reaction leading to cellular blast transformation whereas DLE has a different basis. This suggests that the mechanisms generating and/or maintaining lymphocyte infiltration are different in LP and DLE. LP lesions have been proposed to be due to an increased level of γ-interferon in the affected skin (14).

The mechanisms leading to and maintaining massive lymphocyte extravasation in DLE and LP, respectively, are unknown. So far there are no reports concerning presence of homing-receptors similar to those on high endothelial venule cells (15) on endothelial cells in the skin. However, it is of interest with respect to the mechanisms of lymphocyte accumulation in the skin that adhesion of T-lymphocytes to

both endothelial cells and keratinocytes *in vitro* involves LFA1 molecules on the T-cells and ICAM1 (intercellular cell adhesion molecule 1) on endothelial cells and keratinocytes (16).

According to one hypothesis, the capacity of lymphocytes to infiltrate is linked to activation of the cells to blast transformation (2). The fact that extensive lymphocyte infiltration in LP and DLE in the first case is accompanied and in the second case is not accompanied by IL2 receptor expression, argues against a simple association between activation to blast transformation and infiltrative capacity.

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