Acta Derm Venereol (Stockh) 1985; 65: 398–402

Immunophenotyping of the Dermal Cell Infiltrate in Lichen planus Treated with PUVA

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Naukkarinen A, Väätäinen N, Syrjänen KJ, Horsmanheimo M. Immunophenotyping of the dermal cell infiltrate in lichen planus treated with PUVA. Acta Derm Venereol (Stockh) 1985; 65: 398-402.

To evaluate the immunoregulatory mechanisms in lichen planus (LP) responding to PUVA therapy, the LP skin lesions of eight patients were biopsied before and after treatment with trioxsalen baths and UVA. The percentages of B, T, and MPS (monocyte-macrophage) cells as well as OKT-4$^+$ (helper) and OKT-8$^+$ (suppressor) T cells were calculated. OKT-8$^+$ T cells were the dominating cell type in untreated LP lesions. Following PUVA therapy, the percentage of OKT-8$^+$ T cells decreased very significantly ($p<0.001$) as did also the number of MPS cells. These results suggest that cell-mediated immune mechanisms are involved in the pathogenesis of LP, and T suppressor cells most probably participate in evolvement of the typical LP lesions. T suppressor cells in the LP lesions are reduced after local PUVA therapy, occurring simultaneously with complete healing of the affected skin. *Key words: Lichen planus; PUVA; Lymphocyte subsets; MPS cells.* (Received April 3, 1985.)

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In lichen planus (LP), the characteristic histological feature is a dense, band-like mononuclear cell infiltrate in the upper dermis. This dermal infiltrate consists mainly of lymphocytes, most of them T cells, and some macrophages (1, 2, 3). By defining the T cell subpopulations in LP lesions with monoclonal antisera, predominance of helper/inducer subset over the suppressor/cytotoxic one has been reported (4, 5, 6). Other recent studies, however, suggest that the T suppressor/cytotoxic subset would be responsible for the characteristic degenerative lytic process in the basal keratinocytes of LP lesions (7, 8). A similar process has been observed in the lesions of chronic graft-versus-host reactions (9, 10).

Favourable clinical response has been obtained in the therapy of urticaria pigmentosa and LP using oral methoxsalen photochemotherapy (11, 12) or trioxsalen baths with UVA (13). To assess the role of immune mechanisms in such a response to PUVA the composition of the dermal inflammatory cell infiltrate in LP lesions before and after PUVA therapy is analysed using immunohistochemical methods.

PATIENTS AND METHODS

Patients

Eight patients had lichen planus with diffuse papular lesions as established in biopsy. Six patients were females with a mean age of 51 years (range 31–61), and two were males (40, 41).

Therapy

Pure trioxsalen (Fermion, Finland) was dissolved in ethanol and 50 mg (20 ml alcoholic solution) was added to 150 l of warm water. The bathing time before UVA was 10 min (14). The UVA source was an ordinary UVA cabin (PUVA 22, Astra-Sjuco, Helsinki, Finland) with efficiency of 10 mW/cm^2.

Fig. 1. Untreated LP lesion. Numerous T suppressor/cytotoxic cells (arrows) are seen subjacent to the lytic basal cells of the epidermis (E). Avidin-biotin-peroxidase complex technique with OKT-8, ×1 400.

The therapy was started with 0.10 J/cm^2 of UVA per exposure. This treatment was given 3–4 times a week, and the UVA dose was increased by 25% every third exposure, depending on the patient's reactions to the treatment. The therapy was continued until clear healing of the lesions was achieved.

Immunohistochemical staining procedures

For immunohistochemical studies, each patient was biopsied before and after the PUVA therapy. The skin biopsies were frozen in isopentane cooled with liquid nitrogen, and stored at −70°C until further processed. Cryostat sections of 4μm thickness were stained for B lymphocytes, MPS (monocyte-macrophage) cells, and T lymphocytes as well as for the subsets of the latter. For these purposes alpha-naphthyl acetate esterase (ANAE) (15) staining and monoclonal antibodies using the avidin-biotin peroxidase complex technique were applied. In brief, the 4 μm sections were fixed in cold (+4°C) acetone for 10 min and air-dried at room temperature. Possible endogeneous peroxidase activity was blocked by methanol—hydrogen peroxidase solution, whereafter the sections were incubated for 15 min in each of the diluted monoclonal antisera. Of the monoclonal antibodies used, OKT-3 (Ortho Immunobiology Ldt, Raritan, N. J., USA) reacts with all peripheral T lymphocytes, OKT-4 (Ortho) with helper/inducer cells, and OKT-8 (Ortho) with suppressor/cytotoxic subset (16). Biotinylated rabbit anti-mouse IgG (Vector Laboratories, Burlingame, Ca, USA) ws applied on sections for 15 min, and avidin-horseradish peroxidase (Vector) for another 15 min. The brown product of the peroxidase reaction was developed using 3,3-diaminobenzidine hydrochloride (Sigma Chemical Co, St. Louis, Mo, USA) as substrate for 5 min. Human tonsils or lymph nodes were frozen, cut and processed for positive controls. Similarly stained specimens but the primary antibody omitted were used as negative controls. The positive reactivity for each monoclonal antibody was manifested as a membrane or granular staining of lymphocytes.

In ANAE-staining, the cells displaying a dark brown dot-like cytoplasmic reaction product were considered T cells whereas the MPS cells stained diffusely brown throughout their cytoplasm. The cells remaining negative for ANAE were considered B cells (17).

The percentages of ANAE T$^+$, ANAE M$^+$, ANAE- and OKT-3$^+$ cells were calculated by counting 200 cells at random in the dermal cell infiltrates subjacent to the epidermis. The percentages

Table I. *Composition of the cellular infiltrate in LP lesions before and after PUVA therapy (M ± SEM)*

Cell type	Before PUVA	After PUVA	*p*
ANAE− cells	39.6±6.2	40.0±4.5	p=NS
ANAE T^+ cells	50.9±3.8	53.0±1.6	p=NS
ANAE M^+ cells	16.5±5.9	6.3±1.3	$p<0.001$
OKT-3^+ cells	62.3±4.0	54.9±3.7	p=NS
OKT-4^+ cells	26.2±6.9	79.1±9.3	$p<0.001$
OKT-8^+ cells	69.6±10.1	22.0±3.9	$p<0.001$
OKT-4^+/OKT-8^+	0.6±0.12	2.5±0.93	$p<0.001$

NS = not significant.

of OKT-4^+ (T helper) and OKT-8^+ (T suppressor) cells were counted in relation to the number of OKT-3^+ cells. The OKT-4^+/OKT-8^+ ratio was also calculated, separately in each specimen.

In comparison of the means, Student's *t*-test was used where separately indicated.

RESULTS

Following PUVA therapy, the lesions disappeared completely in all patients with an average total UVA dose of 34.6 J/cm^2 given as 38 UVA exposures. The only side-effect due to this local photochemotherapy was slight irritation of the skin appearing in three patients. Although the skin was cleared after PUVA treatment, the dermal cell infiltrate was markedly reduced, but had not completely disappeared.

The percentages of B, T, MPS, OKT-3^+, OKT-4^+ and OKT-8^+ cells, and the OKT-4^+/OKT-8^+ ratio determined before and after PUVA therapy are presented in Table I. The untreated LP lesions consist of 50–60% T lymphocytes, most of them definable as T suppressor/cytotoxic cells (Fig. 1). In addition, 35–45% B lymphocytes and 10–20% MPS cells are present. After PUVA therapy, no significant changes in the relative number of B or T cells can be observed. A highly significant decline ($p<0.001$) is, however, evident in the number of MPS cells after PUVA. Also the ratio of OKT-4^+/OKT-8^+ cells shows a dramatic change shifting from 0.60±0.12 to 2.5±0.93 ($p<0.001$). In only one patient, there was no decrease in the number of T suppressor cells after PUVA treatment. A slight disaccordance in the numbers of T lymphocytes occurs when counted in sections stained with ANAE and those stained with the monoclonal antibody OKT-3 (Table I).

DISCUSSION

Local photochemotherapy using trioxsalen baths and UVA has proved to be effective in the treatment of psoriasis, urticaria pigmentosa and lichen planus (LP) (13, 14). This was also confirmed in the present study, where the skin lesions of eight patients with papular LP healed completely following local PUVA therapy. The mechanisms responsible for this favourable response to PUVA are not fully understood yet.

In the present study, the inflammatory dermal cell infiltrate contained predominantly T lymphocytes, which had been confirmed earlier (1, 2, 3). There was a difference, however, between the number of ANAE T^+ and OKT-3^+ cells (Table I). This indicates that ANAE does not stain all T cells. Such ANAE-negative T cells have been purified from human adenoids and tonsils in high percentages (18). The percentage of OKT-3^+ T cells in the LP

lesions was close to 60, which is in agreement with the figures (65%) reported by Braathen et al. (2), but somewhat lower than that (75%) by Bjerke (5). No significant changes were observed in the relative proportions of B and T cells following PUVA therapy (Table I).

The MPS cells, that were quite numerous in the untreated LP lesions (10–20%), decreased significantly ($p<0.001$) following PUVA therapy. This seems natural; when inflammation subsides fewer macrophages are needed in the healing skin (19). Furthermore, De Panfilis et al. (20) reported that macrophages are more numerous in the early lesions of LP, their number diminishing as the disease proceeds. The relatively high percentage of ANAE M^+ cells in the LP infiltrates might be partly due to Langerhans' cells, shown to be present in such lesions (4, 9, 10), and to belong to the macrophage population of the skin (21).

In the untreated LP lesions, about 70% of the T cells represent the suppressor/cytotoxic subset (Table I). T suppressor cells have been shown to predominate (20–70%) in the late lesions of LP, whereas in the early lesions, T helper cells outnumber T suppressor cells (22). In contrast, Gomes et al. (10) detected a large number of T suppressor cells in the early lesions of cutaneous LP, and Löning et al. (7), who studied oral LP, reported that the majority of lymphocytes at the epithelial-mesenchymal interface are distinctly labeled by OKT-8 antibody.

The studies on the effects of PUVA on peripheral lymphocytes have yielded controversial results. Long-term PUVA treatment has been shown to alter the function, cell surface markers and distribution of lymphocytes both in vitro and in vivo (23). In another study, however, PUVA is reported to exert no effects on lymphocyte function in vivo (24). In the present study, the dermal cell infiltrate was not completely disappeared after PUVA treatment, although the skin lesions were healed. PUVA therapy did induce dramatic changes in the cellular composition of the *in situ* infiltrate: The T helper/T suppressor cell ratio increased, reaching 2.5 ± 0.93, i.e. about 80% of the OKT-3^+ T cells now representing the helper/inducer subset (Table I).

The abundance of T suppressor cells in untreated LP lesions well explains the degenerative and necrotic processes affecting basal keratinocytes in the epidermis (7, 8, 10). Following the decrease in their number the direct cytotoxic effect of T suppressor cells towards the basal epidermal cells can be diminished as well. In the healing skin, the activity of the inflammatory process in the dermo-epidermal junction is likely to cease followed by evolving fibrosis (19). Obviously, when T suppressor/cytotoxic cells disappear in the lesion, the basal cells remain unattacked and inflammation gradually subsides. Studies where different phases of LP have been investigated suggest that in the first phase of the disease, T lymphocytes in LP lesions interact with MPS and Langerhans' cells (= T cell activation). In the second phase, activated T cells attack and destroy the keratinocytes (9, 19, 24). The present results are in alignment with this concept, emphasising the role of T suppressor/cytotoxic cells in the generation of the LP lesions.

In conclusion, it seems evident that cell-mediated immunity is centrally involved in the pathogenesis of LP, and T lymphocytes belonging to the suppressor/cytotoxic subset are participants in generating the typical LP lesions. This is demonstrated by the results of the present study, where local PUVA therapy lead to complete healing of the affected skin, and at the same time, reversed the OKT-4^+/OKT-8^+ ratio by depleting the LP lesions of their T suppressor/cytotoxic cells.

ACKNOWLEDGEMENTS

This study was supported by Research Grants from the Medical Council of the Academy of Finland (07/014) and from the Finnish Cancer Society. The skillful technical assistance of Mrs Heli Eskelinen and Miss Ritva Savolainen is gratefully acknowledged.

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