

PUVA PHOTOHYPOSENSITIZATION IN POLYMORPHOUS LIGHT ERUPTIONS: EVALUATION OF SYSTEMIC IMMUNOLOGICAL FACTORS

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Abstract. Systemic immunological parameters were monitored in 26 polymorphous light eruption (PMLE) patients prior to, during and after photochemotherapy by either an oral or topical (bath) regimen. Pre-therapy investigations showed a normal immune status as regards the numbers of peripheral T-cells as well as the response of peripheral blood lymphocytes to PHA, ConA and PPD. Measurement of the immunological parameters after four PUVA exposures revealed a statistically significant increase in the lymphocyte PHA responses. After the completion of a PUVA course of some 20 irradiations, normalized PHA responses were found. The total lymphocyte count increased slightly during the treatment but the relative numbers of T- and B-cells changed very little. The immunological changes observed were of only moderate strength and it remains doubtful whether they play any major role in the hyposensitizing effect of PUVA irradiations in PMLE.

Key words: Polymorphous light eruptions; PUVA treatment; T-cells; Suppressor T-cells; Lymphocyte transformation tests

Psoralen photochemotherapy (PUVA) is effective in some skin disorders with a possible auto-immune or allergic etiology, e.g. lichen planus (19), atopic dermatitis (16), and polymorphic light eruption (PMLE) (8). UV-irradiation has distinct effects on the immune system of both experimental animals and man (5). Recent studies have shown that PUVA treatment may affect the peripheral lymphocyte population and the general immune status of the treated patient (17). Little is known, however, about the possible influence of PUVA treatment on the immune status of patients with allergic disorders. This paper reports the results of immunological studies in 26 PMLE patients treated with either systemic or topical PUVA.

MATERIAL AND METHODS

Photochemotherapy

Twenty-six patients with long-standing, recurring polymorphic light eruptions were included in the study. Thir-

teen of the patients received oral methoxsalen (0.6 mg/kg) 2 hours before irradiation, and the other 13 patients were bathed for 10 min in a trioxsalen-water solution (50 mg/150 l) immediately prior to irradiation. The details of the diagnostic criteria, patient characteristics, PUVA dosages and clinical results have been given in an earlier report (11).

Lymphocyte subpopulations

These were determined in the 13 orally treated patients. Heparinized (50 U/ml) venous blood was obtained from each subject between 8 and 10 a.m. Blood samples were taken before starting PUVA treatment (sample I), 24-48 h after the 4th PUVA exposure (sample II) and 24-72 h after the last PUVA treatment, i.e. after 13 to 42 (mean 22) exposures (sample III). The mononuclear cells were isolated on a Ficoll-Isopaque (Pharmacia Fine Chemicals, Uppsala, Sweden) density gradient (2) and washed twice with Hanks' balanced salt solution. All washes were performed at 22°C. After the washes, more than 95% of cells were mononuclear, and their viability exceeded 90% as determined by the trypan blue (0.25%) exclusion test.

T-lymphocytes were estimated by an E-rosette test according to Jondal et al. (12). The number of suppressor cells was estimated by measuring the numbers of theophylline-sensitive E-rosetting cells (E_{s} -RFC) (21). Surface immunoglobulin (SmIg) positive cells were determined as described by Pettersson et al. (20).

Lymphocyte transformation

The samples from orally treated patients and the samples from patients treated with bath PUVA were analysed in two different laboratories, but the time schedules for blood sampling were always as described earlier (samples I-III). Heparinized whole blood from patients in the oral PUVA series was diluted 1:7 with RPMI 1640 (Grand Island Biological Co., Grand Island, N.Y.) and used for lymphocyte stimulation as described earlier (4) with slight modifications. In brief, 175 μ l of a blood-RPMI mixture was placed in the flat-bottom wells of a microtitre plate (Micro Test II, Falcon Plastics, Oxnard, Calif.). Thereafter, the mitogens in 25 μ l of RPMI were added to the chosen wells. Control cultures received 25 μ l of plain medium. The final concentrations of phytohemagglutinin (PHA M; Difco Laboratories, Detroit, Mich.) were 25, 125 and 625 μ g/ml, those of concanavalin A (ConA; Pharmacia Ltd, Uppsala, Sweden) were 5, 25 and 125 μ g/ml and those

Table 1. Effect of oral methoxsalen photochemotherapy on lymphocyte subpopulations in PMLE patients

Sample	Lymphocytes Total	T-lymphocytes				B-lymphocytes	
		E-RFC	(%)	E ₁₈ -RFC	(%)	Smlg	(%)
I (n=9)	2 510 ^a ± 339	1 548 ± 404	61.4 ± 11.4	817 ± 257	32.9 ± 10.9	200 ± 93	9.2 ± 4.6
II (n=11)	3 013 ± 723	1 957 ± 700	63.9 ± 10.7	1 124 ± 557	36.4 ± 10.7	259 ± 143	8.8 ± 4.8
III (n=11)	2 057 ± 894	1 264 ± 649	60.4 ± 8.0	718 ± 396	34.6 ± 10.1	175 ± 73	8.6 ± 2.2
Controls (n=13)	2 826 ± 976	1 857 ± 610	66.3 ± 6.0	819 ± 494	33.4 ± 11.1	222 ± 83	8.2 ± 2.8

^a Mean ± SD.

of purified protein derivative of tuberculin (PPD; Statens Seruminstitut, Copenhagen, Denmark) were 0.01, 1 and 100 µg/ml. The total incubation time for PHA and ConA stimulated cultures was 96 h, and that for PPD, 120 h. Eighteen hours before harvesting, 20 µl of ¹²⁵I-labelled 5-iodo-2'-deoxyuridine (¹²⁵IUdR) (0.25 µCi, sp. act. 90-110 mCi/mg, The Radiochemical Centre, Amersham, England) was added to each well, together with 5-fluoro-2'-deoxyuridine in order to increase the uptake of ¹²⁵IUdR (1). The results were expressed as counts per minute. All counts have been corrected to the reference day of ¹²⁵IUdR. The statistical significance of any differences between responses before, during and after PUVA therapy were analysed by paired Wilcoxon test.

In patients treated with the bath PUVA regimen, the proliferative responses of lymphocytes to PHA and PPD were measured with a modified whole-blood culture method. Heparinized venous blood was diluted 1:12 with RPMI 1640 culture medium, supplemented with 10% pooled human serum from healthy males, and distributed on microtitre plates, 150 µl per well. PHA and PPD were added in 50 µl of medium to obtain a final concentration of 15 µg/ml and 10 µg/ml, respectively. These were concentrations that generally gave maximal stimulations in preliminary tests. Cultures for PHA responses were incubated for 72 h and those for PPD responses for 144 h at 37°C in 5% humidified CO₂ atmosphere, 24 hours before the termination of cultures. ³H-thymidine (Radiochemical Centre, Amersham, England) was added (final concentration 2 µCi/ml). Microtitre plate cultures were harvested on fibre glass filters, and the harvested material solubilized (Soluene 350 containing 10% water) prior to liquid scintillation counting. Median responses of triplicate cultures were used to calculate specific responses to PHA and PPD (median response of stimulated culture minus median response of unstimulated culture). Differences between responses before, during and after the treatment were analysed by paired Wilcoxon test.

RESULTS

Lymphocyte subpopulations

The results of the lymphocyte subpopulation estimations are shown in Table 1. Neither the total

numbers of T-lymphocytes (E-RFC) and B-lymphocytes (Smlg-positive cells) nor the number of suppressor T-lymphocytes (E₁₈-RFC) differed significantly between the patients and normal controls before treatment. During PUVA therapy a non-significant increase in the absolute number of total lymphocytes was observed, but the relative numbers of T- and B-cells changed very little.

Lymphocyte transformation

The results from the lymphocyte stimulation tests of the orally treated patients (patient group A) are given in Fig. 1, and those of the bath-PUVA patients (patient group B) in Fig. 2. In both patient groups, normal pretreatment values for PHA response were obtained, but a statistically significant ($p < 0.01$) rise in the PHA response was seen in the blood sample taken after the 4th PUVA exposure (Figs. 1 and 2). This increase was recorded in 12 of the 13 group B patients and in 6 out of 8 group A patients (5 patients not assayed). By the completion of the PUVA course (Sample III) the PHA responses had returned to pretreatment values (Figs. 1 and 2).

In patient group A, responses to ConA were normal both before and during the treatment, but showed a slight non-significant decrease at the end of the treatment.

In both patient groups a normal PPD response was recorded prior to PUVA therapy (Figs. 1 and 2). The results obtained during treatment differed in the two patient groups. A significant ($p < 0.05$) decrease was found after the 4th PUVA exposure in patient group A, while the opposite was true for patient group B ($p < 0.05$). Post-treatment values for group A were not statistically significantly different from starting values, while the PPD responses in group B were still abnormally high ($p < 0.05$).

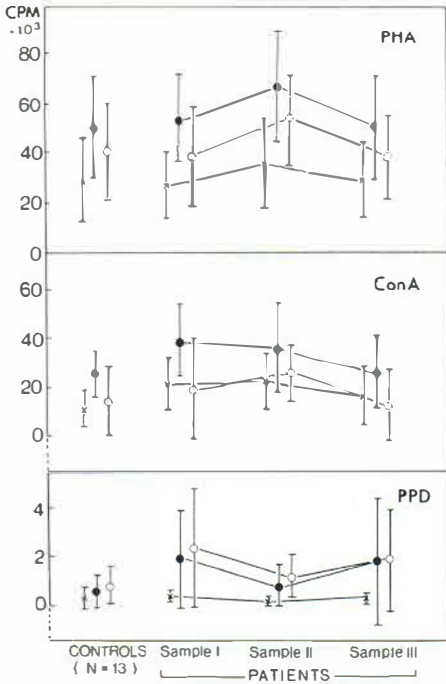


Fig. 1. Lymphocyte transformation by different concentrations of PHA, ConA and PPD before, during, and after oral methoxsalen plus UVA treatment (patient group A). Mean \pm SD ¹²⁵I-UdR incorporations are given. The symbols \times — \times , \bullet — \bullet and \circ — \circ refer to PHA concentrations of 25, 125 and 625 μ g/ml, ConA concentrations of 5, 25 and 125 μ g/ml, and PPD concentrations of 0.01, 1 and 100 μ g/ml, respectively.

DISCUSSION

PMLE is considered to represent a delayed-type hypersensitivity reaction, although the putative irradiation-induced cutaneous allergen has not been identified (10). The pretreatment immunological investigations in the present study indicate that clear-cut abnormalities in lymphocyte subpopulation counts or stimulation responses cannot be demonstrated in PMLE patients, at least not in the clinically inactive stages of the disease. These results are in agreement with and extend the findings of Jung & Bohnert (13) and Menter et al. (15) who described normal lymphocyte PHA responses in patients with PMLE and actinic reticuloid, respectively.

In previous studies both increased (7, 9) and decreased (3, 9) T-cell counts have been reported during and after PUVA therapy in psoriatic patients and healthy controls. Studies on T-lymphocyte subpopulations during PUVA treatment of psoriasis

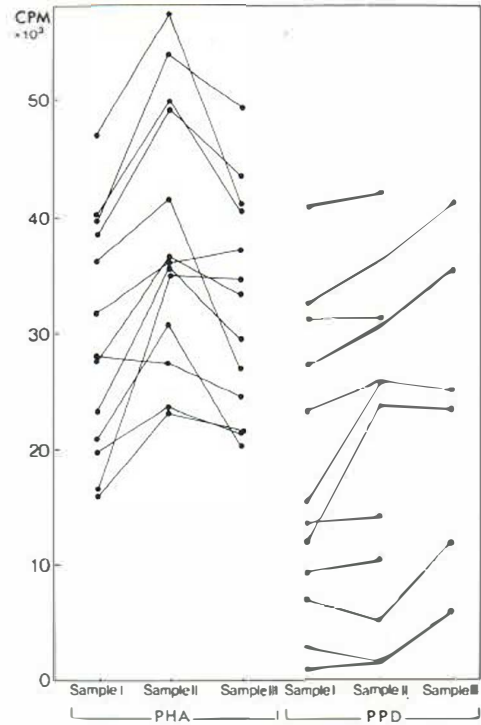


Fig. 2. Lymphocyte transformation by 15 μ g/ml of PHA and 10 μ g/ml of PPD before, during and after topical (bath) trioxsalen plus UVA treatment (patient group B). Values of each individual patient are given.

have shown decreased numbers of helper T-cells (17) and either decreased (7) or normal (17) numbers of suppressor T-cells. Our results showed a non-significant increase during and decrease after the treatment in the total lymphocyte count with very small changes in the relative numbers of T- and B-cells (Table I).

The major positive finding of the present study was the statistically significant rise in PHA responses recorded after the 4th PUVA exposure, though not after some 20 exposures. Although in vitro studies have shown decreased responses of lymphocytes to PHA after psoralen and UVA (14), previous studies with normal controls (18) and psoriatic patients (6, 23) have shown normal PHA responsiveness both after single exposures and after about 15–20 exposures of PUVA. As an increased PHA responsiveness in the present study was found both in the orally medicated and in the psoralen bath treated patient group, the effect is probably linked to a true photochemotherapeutic action of the treatment and not, for example, to a

mere systemic effect of the psoralen drug, as only minute amounts of the psoralen are absorbed through the skin during the 10-min bathing procedure (22).

The increased responsiveness to PPD during local PUVA treatment might be coincidental, as it could not be shown in systemic PUVA. Even in local PUVA the increase was not significant when expressed as an index (observed values compared with responses of 3 control subjects on each day). In comparison, the increase PHA responsiveness was significant ($p < 0.01$) even when expressed as an index.

Taken together, the results of this study indicate that the systemic immunological effects of photochemotherapy in PMLE are of moderate strength and of short duration, and no harmful long-term effects on immune parameters were detected. Our study also rather supports the view that systemic immunological factors do not play any decisive role in the process of PUVA hyposensitization in PMLE.

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