

CELL-MEDIATED IMMUNITY IN CHRONIC POLYMORPHOUS LIGHT ERUPTIONS

*Leukocyte Migration Inhibition Assay
with Irradiated Skin as Antigen*

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Abstract. Homogenates of irradiated and non-irradiated skin from 16 patients with chronic polymorphous light eruptions (CPLE) were examined to detect possible light-induced antigen to cell-mediated immunity. The leukocyte migration agarose tests which were performed disclosed no evidence of migration inhibition in either type of skin. These findings do not support the theory of CPLE being a delayed hypersensitivity reaction against an antigen formed in the skin by actinic radiation.

Key words: Polymorphous light eruptions; Skin antigen; Leukocyte migration test

Chronic polymorphous light eruptions (CPLE) constitute a group of light-induced dermatoses differing in their clinical appearance but having as a common feature an unidentified etiology. It is often suggested that CPLE is caused by an allergic reaction mediated either by specific antibody (6) or cellular immune mechanisms (5, 18). It is believed that radiation generates in the skin of CPLE patients, an antigen which then triggers the allergic reaction (7). To our knowledge, however, there are no published reports establishing whether this is so, i.e. whether antigenic material is demonstrable in the irradiated skin of CPLE patients. We therefore report here the results of our experiments in which irradiated CPLE skin was used as antigen, and autologous leukocytes as detectors of hypersensitivity. The leukocyte migration agarose test was used as the assay system.

MATERIALS AND METHODS

Patients

Sixteen patients with CPLE, representing three of the various morphological variants of the disease (13, 14) and

all registering strongly positive polymorphous light reactions in skin tests with UV-light, were selected for the study at the Departments of Dermatology at the Universities of Oulu and Turku. The clinical data of the patients are given in Table 1. Five volunteers who were not sensitive to sunlight and who suffered from unrelated, limited dermatoses were used as controls. In addition, one patient suffering from light sensitivity due to erythropoietic protoporphyria (EPP) was included. None of the persons tested received any topical medication on the dorsal skin, nor did they receive any internal medication for a period of 2 weeks prior to the experiments.

Preparation of antigen

A 5×5 cm area of skin on the upper back was irradiated for 10 min at a distance of 50 cm with a 300 W Osram Ultra-Vitalux GUR 53 mercury lamp, giving at this distance an output of approx. 720 $\mu\text{W}/\text{cm}^2$ in the 280-325 nm range and 1900 $\mu\text{W}/\text{cm}^2$ in the 325-380 nm range. After an interval of 5 min (in 12 patients and the 5 controls) or 20 min (in 5 patients) skin at the exposed site was taken with a keratome set at minimum depth. Control tissue was obtained from non-irradiated symmetrically situated skin sites. The tissue samples weighed between 36.9 and 139.3 mg. They were homogenized in Tenbroek-type tissue grinders in 140 mM phosphate buffer, pH 7.1, using 1 ml of buffer per 50 mg of tissue sample. After centrifugation for 15 min at 1000 g, the supernatant was collected and used as one antigen. The precipitate was resuspended in the original volume of phosphate buffer and used as another antigen. In 13 of the patients and in the 5 controls, purified protein derivative of tuberculin (PPD), obtained from Central Veterinary Laboratory, Surrey, England, was used as a third antigen.

Preparation of leukocytes

Fifty ml blood samples from the cubital vein were drawn into a heparinized syringe (200 IE/10 ml blood) and allowed to settle in an upright position at 37°C for 60 min. In most cases the blood sedimented spontaneously but occa-

Table 1. Clinical data on the patients in this study.

TTS signifies the smallest amount of sun exposure on a clear summer day capable of precipitating an exacerbation of the dermatosis

Patient number	Sex	Age	Duration of photo sensitivity (years)	Type of photodermatosis	Threshold tolerance to sun (TTS)
1	♀	50	4	CPL.E. vesicopapular type	10 min
2	♂	52	11	CPL.E. vesicopapular type	15 min
3	♂	47	10	CPL.E. vesicopapular type	20 min
4	♀	28	5	CPL.E. vesicopapular type	30 min
5	♂	41	10	CPL.E. vesicopapular type	30 min
6	♂	64	43	CPL.E. vesicopapular type	1 hour
7	♀	38	25	CPL.E. vesicopapular type	1 hour
8	♀	18	9	CPL.E. vesicopapular type	1 hour
9	♂	49	7	CPL.E. vesicopapular type	1-2 hours
10	♂	43	7	CPL.E. vesicopapular type	Several hours
11	♀	58	41	CPL.E. eczematous type	1-2 min
12	♂	58	21	CPL.E. eczematous type	10 min
13	♀	62	6	CPL.E. eczematous type	20 min
14	♂	52	4	CPL.E. eczematous type	1 hour
15	♀	59	2	CPL.E. maculonodular type	20 min
16	♂	47	19	CPL.E. maculonodular type	Several hours
17	♀	24	22	Erythropoietic protoporphyria	15 min

sionally when the sedimentation rate was low, the blood was mixed with 5% dextran (Dextran 150; Pharmacia, Uppsala, Sweden) in RPMI 1640 tissue culture medium (Grand Island Biological Company, Berkeley, California, USA) in the proportion 1:20. The leukocyte-rich plasma supernatant was centrifugated at 150 *g* for 15 min. The remaining cell pellet was washed three times in Hanks' balanced salt solution. The washed leukocytes were divided into five parts (or seven parts, if PPD also served as antigen) and were then resuspended in RPMI 1640 medium containing 10% foetal calf serum to give a final concentration of 6×10^7 leukocytes per 0.2 ml.

Migration inhibition test

Migration inhibition of the leukocytes in agarose medium was studied using the method introduced by Clausen (1, 2). Agarose medium was prepared by mixing agarose (Indubiose[®], supplied by l'Industrie Biologique Française), Auto-Pow (Basal medium Auto-Pow cat. no. 1 A-010, Flow Laboratories, Rockville) and distilled water under heating. The solution was left to cool to +45°C. Glutamine, foetal calf serum (Difco) and sodium bicarbonate were added in such proportions that the final solution contained 0.75% agarose, 10% foetal calf serum and 0.8% Auto-Pow basal medium. Na-penicillin (100 IU/ml) and streptomycin (100 µg/ml) were added and 7 ml of the agarose medium was poured into disposable plastic Petri dishes, diameter 9 cm (Nunc, Denmark). After gel formation, 2 mm diameter wells were cut out of each agarose plate, using a stainless steel punch. One-tenth ml antigen volumes were added to the leukocyte suspension and the same amount of phosphate buffer was added to the leukocyte suspension which served as control. In 13 patients and 5 control persons, 50 µl of PPD and, as control, the

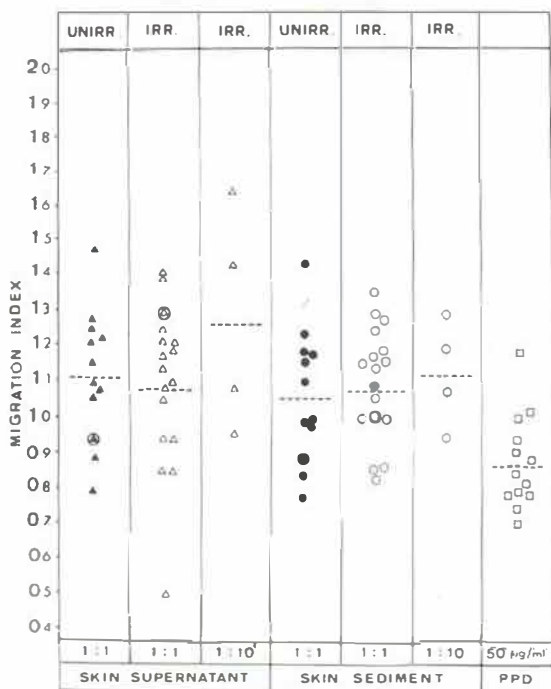


Fig. 1. Results of the leukocyte migration tests for the 16 CPL.E patients, and also for 1 EPP patient whose results are encircled. 1:1 denotes undiluted skin antigen and 1:10 a ten-fold diluted antigen. Unirr=unirradiated, Irr=irradiated.

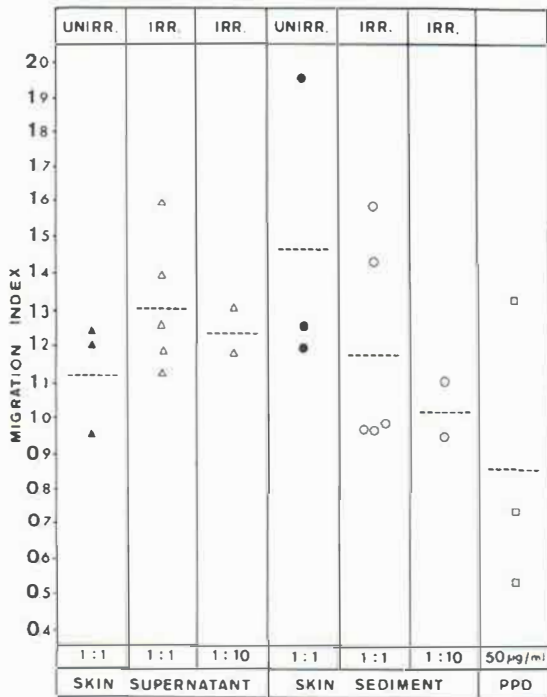


Fig. 2. Results of the leukocyte migration tests for the 5 control persons. Antigen dilutions and abbreviations are the same as in Fig. 1. Absence of data is due to lack of sample.

same amount of distilled water, were added to the leukocyte suspensions. The PPD concentration in the leukocyte suspension was 50 µg/ml. The leukocyte suspensions were incubated with the antigens at 37°C for 30 min, and thereafter the wells in the agarose plates were filled with 5 µl of each suspension. The agarose plates were then incubated at 37°C in 7% CO₂ in air for 18 hours. After incubation the cells were fixed on the bottom of the Petri dishes by submersion with formaldehyde solution. The agarose medium was removed and the cells were stained with 0.1% cresyl violet. Migration areas were measured by planimetry under a projection microscope. All assays were made in triplicate. The degree of migration was expressed as a migration index (MI), i.e. the ratio of the average areas obtained in the cultures containing antigen, vs. the controls. Migration inhibition of 20% or more was considered as the criterion for a positive leukocyte migration test.

Skin tests

An intracutaneous tuberculin test (PPD ITU, State Serum Institute, Copenhagen, Denmark) containing 0.02 µg purified protein derivate of tuberculin was made in 2 controls and in 7 patients. Tuberculin-positive persons were defined as those who developed an induration of 5 mm or more 48 hours after intracutaneous injection of 0.1 ml of the tuberculin solution.

RESULTS

Skin antigens

As seen in Fig. 1, the migration indexes in assays with both irradiated and unirradiated skin antigen show scattering, with most values falling in the 0.9–1.3 range. On the whole, no difference between irradiated and unirradiated skin is revealed, nor is there any conclusive difference between skin supernatant and precipitate antigens. Only one assay with irradiated skin antigen falls within the range of significant migration inhibition (i.e. migration factor ≤ 0.8), and is probably due to a technical failure. The length of the interval (5 or 20 min) between irradiation and sample collection did not influence the results. As seen in Fig. 2, no conclusive differences were noted between unirradiated and irradiated skin samples from control persons. In some samples there was a rather pronounced migration stimulation but, on average, the migration indexes obtained were of the same order as in the patient series.

Tuberculin antigen

The 50 µl/ml PPD concentration induced significant migration inhibition (20% or more) in 2 controls and in 6 patients. As shown in Table II, the in vitro results tallied fairly well with the results from the tuberculin skin tests. Probably, the migration inhibition would have been more marked, and additional positive results would have been recorded, if the PPD concentration had been higher, e.g. 100 or 500 µg/ml, a point recently stressed by Clausen (3). Furthermore, the tuberculin skin test might have been positive in some additional cases if higher concentrations had been tried (10 or 100 TU).

Table II. Comparison of the results of migration inhibition assays and of intracutaneous tests, using PPD as antigen

Migration index	Mantoux ITU (mm)
Patients	
1.17	0
0.99	0
0.93	12
0.80	21
0.78	0
0.78	15
0.77	18
Controls	
1.32	0
0.73	8

DISCUSSION

Several well known clinical facts strongly support the concept of an allergic pathogenesis of CPLE. Thus, the condition appears to be an acquired one, since it may appear at any age (e.g. 4, 8, 13) and since intense light exposure seems to be a predisposing factor (13). Also, the light sensitivity tends to become more pronounced during the first years after its appearance and often extends to skin areas initially not sensitized (13). In addition, the flare of previously involved skin sites after exposure of uninvolved skin (15) is often cited as evidence of an allergic nature of CPLE. Indicative of a cell-mediated immune mechanism, rather than an antibody mediation, are the long (up to 2 days) lag between light exposure and the appearance of the eruption, and the similarity of both the microscopic picture (4) and the macroscopic features of polymorphous light eruption to those of allergic contact dermatitis.

Passive transfer of CPLE with the serum from 2 of 6 patients was accomplished by Epstein (6), implying the presence of either specific antibodies or specific proantigen in the serum of CPLE patients. However, other investigators have failed to confirm this finding (15, 18). Any cell-mediated hypersensitivity should, on the other hand, be transferable with white blood cells, but such attempts have so far been unsuccessful in CPLE (5).

Useful *in vitro* tests for cell-mediated hypersensitivity include the migration inhibition test and the lymphocyte transformation test. The former has proved to be an efficient detector of the immunologic mechanism involved in solar eruptions caused by salicylanilide contact photoallergy (10). If, as Epstein (6) postulated, light acts in CPLE by producing in the skin a photo-product which acts as an antigen, this ought to be detectable in irradiated skin by the leukocyte migration test used in the present study. As we were unable to detect any inhibition, it is possible that such an antigen is not formed in CPLE skin. Another explanation for our negative results could, of course, be technical failure. Any systemic error in our system would seem to be ruled out as the control assays for PPD sensitivity did work well, and also because we found the method to correlate well with skin tests in assays for dermatophyte sensitivity (9). Technical failure to demonstrate specific antigen in irradiated CPLE skin might, however, be related to the following variables: light source and dose, interval

between irradiation and collection of skin sample, the technique of obtaining and processing the skin sample, and the antigen concentration used. The light source and dose must be considered adequate, as the technique, in every CPLE patient of this study, induced a pronounced polymorphous light reaction in the tested site 1–4 days after exposure. Intentionally, two intervals (5 and 20 min) between skin irradiation and sample collection were used, though theoretically, antigen could have been missed if it was very short-lived or was formed later than 20 min after irradiation. The keratome skin sample contained mainly epidermal tissue and therefore if the antigen formation took place in the dermis this could possibly have remained undetected in our study.

To rule out any unspecific effect on the leukocyte migration of the undiluted skin extract, a ten-fold dilution of the antigen was used in some experiments in addition to the undiluted one, but no consistent differences were noted. Of course, there still remains the possibility that the antigen concentration in the undiluted skin extract was too low to give any significant migration inhibition.

Two reports on the use of similar techniques in the investigation of CPLE patients have been published (11, 16), but in neither study was skin used as antigen. Horkay & Mészáros (11), measuring lymphocyte transformation in irradiated white blood cells of CPLE patients, did not state if they suspended the leukocytes in autologous serum. It is difficult to see the point in irradiating white blood cells (instead of skin tissue) and we find it difficult to interpret the very moderate increases in blastogenesis and thymidine incorporation in irradiated CPLE lymphocytes as indicative of anything. Raffle and co-workers (16) irradiated CPLE patients' leukocytes and/or serum and measured the effect on both transformation and leukocyte migration. From their negative results the conclusion can be drawn that no specific proantigen is contained in the serum of patients suffering from CPLE.

Thus, the findings of this study, as well as previous failures to transfer CPLE sensitivity with leukocytes (5) do not support the clinical impression of CPLE as being an expression of cell-mediated allergy. The statement of Ryan (17) that CPLE may be an extreme variant of a normal cutaneous reaction pattern to actinic radiation could be an alternative explanation, supported by the fact that abortive forms of the disease seem to be readily produced in

a large proportion of the population, provided the stimulus is strong enough (19). Additionally, atopic skin reactivity mechanisms have been proposed as a possible common factor in CPLE (12).

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