Acta Dermatovener (Stockholm) 51: 268–270, 1971

A STUDY ON LYMPHOCYTE TRANSFORMATION IN LIGHT DERMATOSES

Preliminary Report

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Abstract. Allergy to UV light was studied in patients with polymorphous light eruption (PLE) by means of lymphocyte transformation. Data obtained by investigation of the morphological changes and the synthesis of DNA as well as RNA indicated tentatively that blastogenesis after UV light irradiation occurred only in those cultures derived from patients with PLE while the phenomenon was not observed in the cultures derived from healthy persons and patients with porphyria cutanea tarda. The results cannot be explained by the "repair mechanism".

It was first Duke, and later Epstein, who called attention to the photoallergic pathogenesis of chronic polymorphous light eruption (PLE) (4). In this disorder UV-light produces a metabolite in the skin acting as an antigen and inducing antibody-production (5). The nature of the pro-antigen and the antigen developed by irradiation is unknown so far, nevertheless much indirect evidence supports this hypothesis (5, 11). Some data indicate that the allergy is manifested as a delayed hypersensitive reaction (6, 11, 16). The detection of the lymphocyte transformation brought about by a specific antigen stimulus represents a valuable method for the investigation of delayed hypersensitivity (1). In the present study we have intended to use this modern procedure to investigate, *in vitro*, the photoallergy in PLE. We were unable to find any data about it in the literature available.

MATERIAL AND METHODS

The persons investigated: 13 patients with PLE, aged 10-63, 8 patients with porphyria cutanea tarda, representing a photodynamic dermatosis of non-allergic pathomechanism; 14 healthy control persons. The cultures of lymphocytes derived from these persons were prepared under sterile conditions according to Heitmann's modified method (9). The cell count was: $1-2 \times 10^6$/cult. A culture stimulated by PHA-P Difco was used as a positive control, and a non-irradiated one as a negative control. After preparing the cultures we irradiated them with with UV-light by means of a quartz lamp (type Medicor Q 250 W) from a distance of 30 cm for 1, 2, 5 and 10 min, with the total spectrum of the lamp or with the spectrum above 3 200 Å. (The output of the lamp: 2 000–2 800 Å: 47.37 μW/cm^2; 2 800–3 150 Å: 150.65 μW/cm^2; 3 150–4 000 Å: 110.85 μW/cm^2, from a distance of 1 m).

Then the cultures were incubated at 37°C for 3–5 days according to the further elaboration. The estimation of the morphological changes was carried out in 96–120 hour cultures by Heitmann's method (9). For estimation of the number of lymphoblasts 500–1 000 mononuclear cells were counted.

For measuring the synthesis of DNA, 96-hour cultures were supplemented with 0.4 μCi/cult. ^{14}C-2-thymidine, and for the synthesis of RNA, 72-hour cultures were supplemented with 0.2 μCi/cult. ^{14}C-2-uridine (spec. act. of both: 44 mCi/mM). After incubating for 5 hours, cultures were filtered on membrane filters (Sartorius MF 100), washed successively with saline and 5% ice-cold TCA. Radioactivity was measured in a dioxane-based scintillation fluid by means of an NZ-137 liquid scintillation spectrometer (Gamma, Budapest). Samples were counted < 3% counting error.

RESULTS

Data of the morphological changes in the 96-hour cultures after a 1, 2, 5 and 10 min UV-irradiation, carried out once on each of days 1–4, are shown in Table I. The values represent the percentage of lymphoblasts considered normal below 5%. The ratio of number of the lymphocytes found in the irradiated and non-irradiated cultures was considered positive above 1.5. It is noteworthy that in non-irradiated cultures derived from almost every case of PLE the per centage of lymphoblasts was higher than the normal 5%.

Table I. *The lymphocyte transformation* (A) *of control persons,* (B) *of patients with PLE, and* (C) *of patients with porphyria cutanea tarda in per cent on the basis of cellmorphology*

Q–,1.2 ... = exposure time of UVA-irradiation in minutes; *PHA* = stimulation with phytohaemagglutinin P

Case	PHA	Q–	Q,1'	Q,2'	Q,5'
A					
	20	2.–	1.5	–	–
	28	5.6	–	5.3	–
	25	2.6	–	3.8	–
	18	3.1	–	4.–	–
	38	3.3	–	–	3.1
B					
1	26	8.6	–	11.7	13
2	30	8.1	16	15.–	–
3	21	7.–	–	8.9	–
4	16	7.4	14	15.–	–
5	22	2.–	–	11.–	–
6	38	5.8	8.3	–	–
C					
	25	3.5	4.–	4.–	–
	32	4.8	4.9	4.5	–
	17	4.	3.8	4.3	–
	29	3.1	1.9	3.3	–

Table II. *^{14}C-2-Thymidine incorporation into the DNS-fraction of cultured lymphocytes* (*A*) *of control persons,* (*B*) *of patients with PLE in* $(I/N) \times 100$

Q 1,5 ... = exposure time of UV-irradiation in minutes; *PHA* = stimulation with phytohaemagglutinin P; *A* = irradiation with UVA, *B* = irradiation with the total spectrum of the lamp

	1:Q_1		2:Q_5		3:Q_{10}		4:Q_1		5:Q_2	
Case	A	B	A	B	A	B	A	B	A	PHA
A										
	102	74	88	83	–	–	–	–	–	–
	103	133	111	142	91	88	–	–	–	205
	73	111	108	108	98	93	–	–	–	–
	75	*220*	60	94	–	–	–	–	–	–
	–	–	–	–	–	–	84	–	91	473
	–	–	–	–	–	–	66	–	86	–
B										
7	–	97	–	*197*	–	–	132	*229*	125	268
8	–	134	–	110	–	92	–	–	–	220
9	*162*	–	67	–	–	–	–	–	–	–
10	115	–	*194*	–	79	–	–	–	–	–
11	*215*	*182*	*172*	–	–	–	–	–	–	365
12	–	122	–	*198*	–	–	–	–	–	–
13	–	–	–	–	–	–	*208*	–	*182*	–
4	66	63	–	103	53	68	68	*184*	67	–

The lymphocytes of all 3 groups (PLE, PCT and control) reacted to PHA stimulus with a transformation of 16–38%.

The incorporation of ^{14}C-2-thymidine and ^{14}C-2-uridine into the DNA, respectively RNA fractions of the lymphocytes is demonstrated in Tables II and III. The data show the ratio between the count per minute (CPM) of irradiated and non-irradiated cultures, multiplied by 100 ($(I/N) \times 100$). The result was considered positive above 150 (13). One group of cultures (1, 2, 3) was irradiated only once within the first hour for 1, 5 and 10 min with UVA ("A"), respectively with the total spectrum of the lamp ("B"), and another group (4, 5) was treated once on each of days 1–4.

The cell count of the cultures was found to be lower at the end of the procedure than initially, but there was no significant difference between the samples derived from the same person treated in different ways, except in cases treated with the total spectrum for 10 min (toxic effect of UV-light).

DISCUSSION

It would seem to be interesting to investigate whether the light allergy in PLE appearing as a delayed hypersensitive reaction may be demonstrated by means of the method of lymphocyte transformation and whether the reaction of lym-

Table III. *^{14}C-2-Uridine incorporation into the RNS-fraction of cultured lymphocytes* (*A*) *of control persons,* (*B*) *of patients with PLE, and* (*C*) *of patients with porphyria cutanea tarda in* $(I/N) \times 100$

Q 1,5 ... = exposure time of UV-irradiation in minutes; *A* = irradiation with UVA; *B* = irradiation with the total spectrum of the lamp

	1:Q_1		2:Q_5		3:Q_{10}	
Case	A	B	A	B	A	B
A						
	115	50	83	*270*	–	–
	62	89	60	–	–	–
	73	139	65	133	–	–
B						
6	*570*	*1 300*	*450*	*1 000*	–	–
7	–	*223*	–	*289*	–	–
9	63	111	100	*220*	50	98
11	–	104	147	*433*	–	–
C						
	100	115	100	100	–	–
	125	85	75	75	–	–
	91	89	95	150	–	–
	82	102	128	*153*	–	–

phocytes to UV light reflects the difference of pathomechanism between the photoallergic and photodynamic disorders. Because the pathogenic wave length in PLE involves UVA besides UVB, the irradiation of the cultures derived from our patients with PLE was carried out with both UVA and the total spectrum of the lamp (15). To exclude the possibility that the increased thymidine incorporation observed in the present study might have resulted from the so-called repair mechanism, we carried out experiments on uridine incorporation (3, 7, 8, 14). We do not know of any data concerning UV-induced RNA damage and consequential uridine incorporation. Our morphological observations verify an incorporation resulting from blastogenesis in the parallel cultures as well.

Jung's experiments prove that UV-light is able to induce a blastogenesis with the drug Jadit® (*N*-butyl-2-hydroxi-4-chlorbenzamid—Hoechst A.G., Frankfurt, BRD) in cultured lymphocytes derived from patients with photocontact dermatitis after being sensitized with Jadit® (10).

Our data obtained by observation of both morphological changes and of DNA and RNA synthesis (to reduce the deficiencies of the two methods, (2, 12)), seem to indicate tentatively that UV-light induced lymphocyte transformation of an intensity greater than normal in the cultures of most of the patients with PLE. Their scales are almost alike according to the results obtained by observation of the cellular morphology and the synthesis of DNA. At the same time this phenomenon cannot be observed in the irradiated cultures derived from healthy persons and patients with porphyria cutanea tarda of photodynamic pathogenesis.

Further positive explanations cannot be given for the time being.

ACKNOWLEDGEMENT

We are grateful to Dr Kovács for performing the measurements of the radioactivity.

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Received January 11, 1971

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