

Early Detection of Epidermal Dust-like Particles in Experimentally UV-induced Lesions in Patients with Photosensitivity and Lupus Erythematosus

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Dust-like particles, producing a specific fine-speckled, epidermo-subepidermal direct immunofluorescence staining pattern, have been associated mainly with subacute cutaneous lupus erythematosus (LE). Under experimental conditions the appearance of immunoglobulins along the basement membrane in ultraviolet (UV) light-induced lesions has been reported as a late phenomenon.

In this study, photoprovocations with UVA and UVB light were carried out in 16 photosensitive patients with discoid ($n=13$), subacute cutaneous ($n=2$) or systemic LE ($n=1$) and serial biopsies from UV-induced lesions were processed for direct immunofluorescence. A specific, fine-speckled epidermal staining was detected within 7 to 14 days after UV provocation in 7/16 of the patients; in the majority of those patients associated with anti-SSA antibodies and discoid LE without systemic manifestations of their disease. Key words: UV provocation; immunofluorescence; anti-SSA antibody.

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The pathomechanism of skin lesions in lupus erythematosus (LE) is still unclear. Induction of new antigens, or redistribution of autoantigens that are normally sequestered within epidermal cells, has been proposed as a possible mechanism by which ultraviolet (UV) light initiates or exacerbates cutaneous LE (1). Enhanced expression of SSA antigens on the cell surface membrane of patients with LE may in part explain the photosensitivity in these patients (2). Conflicting results have been reported on the clinical association between photosensitivity in LE patients and the presence of circulating anti-SSA antibodies (3, 4).

Dust-like particles (DLP), a specific fine-speckled, epidermo-subepidermal direct immunofluorescence staining pattern, have been described by several authors (5–7) and have been associated mainly with subacute cutaneous LE (SCLE). This particular staining was found to correlate with the presence of anti-SSA antibodies (8). Under experimental conditions, i.e. after UV provocation, the appearance of immunoglobulins in UV-light-induced lesions has been reported as a late phenomenon (4, 6, 9) and mainly along the basement membrane (BM).

Using UVA and UVB, the aim of this study was to investigate the early immunofluorescence findings in experimentally induced skin lesions in a group of photosensitive LE patients (10, 11).

MATERIALS AND METHODS

Sixteen patients with a clinical, serological and histopathologically confirmed diagnosis of discoid LE (DLE) ($n=13$), or SCLE ($n=2$) and systemic LE (SLE) ($n=1$) were included. All of these patients had reported light sensitivity in a questionnaire (11). Two healthy females without any known photosensitivity served as controls (JL, SF). With the exception of the SLE patient, who used perioral corticosteroid corresponding to 2.5 mg prednisolone per day, the patients had no internal medication for their skin disease at the time of testing. The study was approved by the Ethics Committee of Karolinska Hospital.

Photoprovocations

Provocations were performed in accordance with a protocol described previously in detail (10). In brief, the patients were photo-tested with UVB and UVA, and the minimal erythema doses (MED), defined as a barely perceptible erythema, were assessed after 24 h. The different light sources are listed in Table I. Two to three multiples of the MED for UVB and UVA, respectively, were then delivered daily for 3 consecutive days at 5×8 cm fields on uninvolved upper back. The aim was to maintain a slight erythema of the skin for several days. If a pathological reaction appeared, provocations were stopped. When MEDs for UVA could not be assessed (light-testing produced only pigmentation), provocations were made with the maximum dose for UVA, 75–100 J.

Serial 4-mm punch biopsies were taken from UVA- and UVB-induced lesions and single biopsies from non-lesional skin. The lesional biopsies were obtained when a pathological reaction was first seen, then on days 3, 7 and weekly until the lesions disappeared, as described in detail elsewhere (10). Biopsies were cut in half and processed for direct immunofluorescence in accordance with conventional techniques (12), and commercially available FITC-conjugated sheep-anti-human antibodies against IgG, IgM, C1q and C3 were used (Dakopatts, Copenhagen, Denmark). Microscopic examination was done in a Leitz Laborlux D epi-illumination fluorescence microscope on three different occasions.

Sera from all of the patients and controls were examined for the presence of antibodies against anti-nuclear antigens (ANA) with a con-

Table I. UV sources in phototests and provocations of 16 patients with cutaneous LE

UVB	Osram high-pressure Xenon-arc lamp (XBO 150 W) in a Zeiss microscope lamp housing with a quartz collector to produce a round, bright spot, 1.5 cm in diameter. The lamp was equipped with a Schott WG 295 filter. Emission spectrum 250–400 nm. The distance from the filter holder to the skin was 20 cm.
UVB	Waldman UV 1000 Cabin with UV6 bulbs, main emission spectrum of 290–370 nm, to produce a bright field 5×8 cm.
UVA	UVASUN 3000 (Mutzas Co., Munich, Germany), a high-pressure metal halide lamp with a main emission spectrum between 340 and 400 nm.

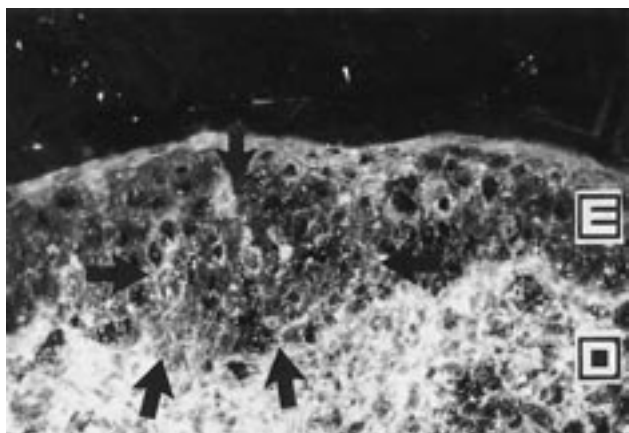


Fig. 1. Positive immunofluorescence with anti-IgG in lesional skin 3 days after first UVA exposure in a female patient with SCLE diagnosis and anti-SSA antibodies (patient BA). Note discrete, fine-speckled mainly cytoplasmic staining (DLP). E: Epidermis, D: Dermis, arrows indicate area with maximal intensity of DLP staining. (FITC-conjugated sheep antihuman antibodies against IgG, $\times 40$.)

ventional indirect IF technique using rat liver as substrate. Antibodies against SSA and SSB were also examined in all patients by immunodiffusion using a commercial kit (Immuno Concepts, Inc., Sacramento, CA, U.S.A.)

RESULTS

Pathological skin lesions were induced by both UVB and UVA. Seven of the 16 patients revealed a discrete, fine-speckled, mainly cytoplasmic, epidermal staining in lesional skin (DLP,

Fig. 1) during the first 2 weeks after artificial UV-light provocation; four with anti-C1q, two with anti-IgG and one with both antibodies (Table II). Five out of the seven patients were anti-SSA positive and four of these five revealed DLP within 7 days after the first UV-light provocation (BA, TE, AH, MC). A similar staining was also detected in non-lesional sun-exposed skin in four SSA-positive patients. One of them (MC) had a high titre of ANA with a granular pattern and showed a nucleus-associated pattern in biopsies from both lesional and non-lesional skin. Six of the seven patients with DLP had the diagnosis of DLE, four of them were anti-SSA positive.

In four patients (UD, LA, AF and KI), linear basal membrane (BM) deposits of anti-IgM were seen in lesional skin within 14 days from UV-light provocation, in two of them also in non-lesional skin (UD, AF). In addition to linear BM deposits, one patient (KI) also showed linear C3 deposits. Three of the four patients with linear deposits were DLP-negative, but one patient (UD) also showed DLP in lesional skin. In the remaining six patients, no specific staining was detected in biopsy specimens taken from photoproved lesions during the first 2 weeks. Only one of these patients was anti-SSA positive.

The two patients with SCLE as well as the one with SLE were all anti-SSA positive as well as four out of thirteen patients with DLE diagnosis. Five patients with DLE were ANA-positive; however, only one of them (MC) had a titre exceeding 1/100.

DISCUSSION

Both *in vivo* and *in vitro* experiments have shown that the expression of SSA antigens can be of importance for the development of light-induced lesions in LE. It is possible that the

Table II. Epidermal IF-staining in lesional and non-lesional skin in photosensitive patients with LE-diagnosis within 2 weeks after artificial UV provocation (UVA and UVB) correlated to previous diagnosis, presence of ANA and anti-SSA antibodies and time after first of 3 consecutive days of UV provocation

Pat./Age/Sex	Diagn	ANA (+/-)	SSA (+/-)	DLP				BM Linear dep			
				L	T	UV	NL	L	T	UV	NL
BA/55/F	SCLE	-	+	IgG	4	A	C1q	-			-
UD/45/F	DLE	-	+	C1q	7	B	-	IgM	7	B	IgM
TE/49/F	DLE	-	+	IgG	6	A,B	IgG	-			-
AH/72/F	DLE	+	+	C1q	3	B	IgG	-			-
MC/41/F	DLE	+	+	IgG,C1q	9	A,B	IgG,C1q	-			-
ALE/65/F	DLE	-	-	C1q	6	B	-	-			-
BT/50/M	DLE	+	-	C1q	8	B	-	-			-
NFS/36/F	DLE	-	-	-			C1q	-			-
LA/52/F	SLE	-	+	-			nd	IgM	9	B	nd
AF/54/F	DLE	+	-	-			-	IgM	6	A,B	IgM
KI/30/F	DLE	-	-	-			nd	IgM,C3	6	B	nd
MJ/50/F	SCLE	-	+	-			-	-			-
KH/67/F	DLE	+	-	-			-	-			-
GWL/57/F	DLE	-	-	-			-	-			-
BG/65/M	DLE	-	-	-			nd	-			nd
RL/50/F	DLE	-	-	-			-	-			-
JL/24/F	-	-	-	-			nd	-			nd
SF/29/F	-	-	-	-			nd	-			nd

+ = detectable, - = undetectable, nd = not done. Diagn: diagnosis, DLP: Dust-like particles, L: lesional, T: time (days) after first UV-provocation, UV: ultraviolet wavelength (UVA or UVB), NL: Non-lesional (sun-exposed), BM: Basal membrane, Dep.: deposition.

speckled epidermal staining we and others have described is part of the immunopathological process in the development of LE lesions. LeFeber et al. (13) originally showed that UV-light-irradiated, cultured human keratinocytes bind IgG antibodies from the sera of LE patients with monospecific anti-SSA activity. Furthermore, enhanced binding of IgG autoantibodies to the cell surface membranes of UVB irradiated SLE keratinocytes was reported by Golan et al. (2). *In vivo*, granular deposition in the epidermis was seen when anti-Ro (SSA) serum was injected i.v. into nude mice with grafted human epidermis (14). Furukawa et al. (15) used specific anti-SSA and anti-SSB probes and demonstrated granular IgG-staining both *in vitro* on cultured keratinocytes after UVB irradiation, and *in vivo* in the roof of suction blisters from UVB-irradiated healthy human skin.

Our finding of DLP *in vivo* after photoprovocation in 7/16 patients, 5/7 of whom were anti-SSA-positive, implies an association between the presence of anti-SSA antibodies and the occurrence of DLP in direct immunofluorescence. Nieboer et al. in the original description of DLP (5) could not show a significant association between the presence of DLP and the finding of anti-SSA antibodies in their series of 35 patients with SCLE. Consequently, they considered immunofluorescence of limited value as a diagnostic tool with a low sensitivity, <30% in SCLE. However, in 1992 both Valeski et al. (8) and David-Bajar et al. (16), found a strong association between DLP in biopsies from lesional skin in patients with SCLE and presence of anti-SSA antibodies, and considered direct immunofluorescence as a useful test for SCLE.

In the present study, DLP was detected within 2 weeks in photoproved lesions in 7/16 of the patients; in four patients as early as within 7 days (BA, TE, AH, MC). All four patients were SSA positive. Patients BA and LA also developed a histologically distinct LE lesion within 1 week after UV provocation (data not shown). In previous studies, several authors have stressed that a positive direct immunofluorescence is a late phenomenon (4, 9). Kind et al. (4) did not find immunoglobulin deposition in biopsy specimens taken from lesions less than 6 weeks after phototesting, whereas Velthuis et al. (6) reported one patient with a positive immunofluorescence finding during the first 7–10 days after light provocation. Only 4 of our patients revealed linear deposits of IgM at the BM, corresponding to a positive lupus band test, in the first 2 weeks after UV-light provocation. It is possible that the appearance of a positive lupus band test is mostly a late phenomenon.

Granular epidermal staining with anti-C1q was detected in lesional skin in five out of seven patients in our study. A similar finding was reported by Nieboer et al. (5), who suggested that this points to an early step in the activation of the complement cascade and that it could imply that DLP are *in situ* formed immune complexes.

Interestingly, altogether four of the patients with DLE were anti-SSA positive, and all of them showed DLP.

In three patients with anti-SSA antibodies, a fine-speckled IgG-staining was detected in non-lesional skin and all three developed DLP in induced lesions. One anti-SSA-negative patient revealed a weak C1q-staining in non-lesional skin. Velthuis et al. found that 2 out of 16 patients with DLE and SCLE showed IgG DLP also in clinically normal skin (6); however, as in our study they obtained non-lesional biopsies from sun-exposed skin (i.e. extensor aspect of arm).

We have shown that in experimentally induced UV-provoked lesions in photosensitive patients with cutaneous LE a specific, fine-speckled epidermal staining can be detected *in vivo* within 2 weeks after UV provocation in immunofluorescence biopsy specimens, and that the majority of patients with this particular staining have anti-SSA antibodies.

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