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Immunohistopathology of Light-induced Skin Lesions in Lupus Erythematosus

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Recently we have been able to induce pathological skin reactions with UVB, UVA and visible light in patients with lupus erythematosus (LE). The pathological skin reactions had the appearance of spontaneously developed LE lesions. In the present study, using patients with polymorphic light eruption as controls, we subsequently investigated what types of immunohistochemical abnormalities were found in these lesions. It was shown that in the induced skin lesions, phenotypically similar inflammatory cells were found as in spontaneously evolved lesions. Granular deposits of immunoreactants, as found in most spontaneously evolved LE lesions, occurred in 12 out of 16 LE patients 7–10 days after onset of the artificial irradiation. The dermal infiltrates in light-induced LE lesions differed mainly from those in polymorphic light eruption, by the amounts of CD1+ cells (Langerhans' cells). In polymorphic light eruption, the relatively large amount of these cells suggests an active migration of antigen-presenting cells, a mechanism apparently not operative in LE. Our results underline the importance of the pathogenic action of light in LE. ***Key words: Immunofluorescence microscopy; Infiltrate analysis; Immunoglobulins; Complement; Photosensitivity; Killer cells; Macrophages.***

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Light sensitivity is an important clinical characteristic of the various forms of lupus erythematosus (LE). However, its pathophysiological basis remains obscure. Experiments with single and repeated UVB exposures induced both LE-like skin lesions and abnormally prolonged persistence of erythema (1–5). Recently we have been able to induce clinically LE-like skin reactions in patients after repeated irradiation with UVB, UVA and visible light (6). In the present study we have investigated whether this form of artificial irradiation of the skin with defined spectra also induces LE-like immunofluorescence and infiltrate characteristics. To this end, 16 patients with different forms of LE, together with 6 patients with polymorphic light eruption serving as controls, were investigated. The results are interpreted in the light of the prevailing theories on the pathophysiology of UV-induced skin changes in LE.

MATERIAL AND METHODS

Patients

Sixteen LE patients participated in this study. Based on clinical, histological and immunofluorescence (IF) features (7–9) they were diagnosed as having chronic discoid LE (DLE; 7 patients), subacute cutaneous LE (SCLE; 7), and systemic LE (SLE; 2). Three patients suffered from dryness of mouth and eyes; one of them was diagnosed as subject to Sjögren's syndrome. One patient received oral prednisone (5 mg) and azathioprine (50 mg), all others were free from oral medication. Twelve of the 16 patients (5 DLE, 6 SCLE, 1 SLE) showed an abnormal response to the UVB phototest procedure (outlined below), 10 also to UVA and 2 also to visible light (6). Six patients with polymorphic light eruption served as controls; all reacted abnormally to UVA and 2 also to UVB. All patients gave their informed consent.

Phototests

A detailed description of the phototesting procedure has been described elsewhere (6). In short: photosensitivity for UVB, UVA, and visible light was tested with respectively: a fluorescent tube (Philips TL 20W/12), emitting a continuum ranging from 280–360 nm, with a maximum around 305 nm; a high-pressure metal-halide source (UVASUN 3000, Mutzhas) with selective filtering to obtain high irradiance in the range 330–460 nm without any measurable UVB; and a super high-pressure mercury lamp (Philips SP500 W) with special cut-on filters for irradiance in the visible spectrum. Average MED values were 160 mJ/cm^2 for UVB, 27.1 J/cm^2 for UVA and 210 J/cm^2 for visible light. Patients were repeatedly exposed on consecutive working days until pathological reactions occurred. The aim of every exposure was to induce a marked erythema, which was bearable by the patient. If no pathological reaction had appeared after six exposures, the test would be stopped and considered negative. Only papular and/or red-scaling (LE-like) infiltrated reactions were considered pathological, whereas erythema and edema were not. Test areas measured 40–60 cm^2 and tests were performed on arms or back.

Immunohistochemistry

Infiltrate analysis. A panel of monoclonal antibodies was used to identify the different cell types in the inflammatory

Table I. *Dermal presence of different cell types in biopsies of provoked LE and polymorphic light eruption (PLE) (figures represent median values)*

	LE (17[a])		PLE (7[a])	
Monoclonal antibody	Interface	Perivascular	Interface	Perivascular
leu 4 (CD3)	4+	4+	4+	4+
leu 3a (CD4)	4+	4+	2+	4+
leu 2a (CD8)	2+	2+	2+	2+
M718	3+	1+	2+	2+
leu M1 (CD15)	1+	±	–	±
OKT6 (CD1)	1+	1+	3+	3+
leu 7	±	±	–	±
leu 11b (CD1)	–	–	–	–
leu 14 (CD22)	–	±	–	–

– = negative, ± = < 1% positive, 1+ = 1–5% positive, 2+ = 6–25% positive, 3+ = 26–50% positive, 4+ = 51–75% positive, 5+ = > 75% positive.
[a] Number of biopsies studied.

infiltrate in 24 biopsies from phototest-sites on 15 patients: 7 biopsies in 6 patients with polymorphic light eruption, 10 in 5 DLE patients, 6 in 3 SCLE patients and 1 in 1 SLE patient. Working dilutions were established by tests on human skin and tonsils and on precipitations after vortex of peripheral blood leukocytes. All biopsies were obtained 7–10 days after onset of the phototests from clinically abnormal reactions. The biopsies were instantly frozen in liquid nitrogen and kept below –70°C until further processing. Eight-µm thick cryostat sections were cut and fixed in acetone. A 3-stage immunoperoxidase reaction was performed: (1) appropriate monoclonal antibody, (2) peroxidase-conjugated rabbit anti-mouse (1 : 40; DAKO), (3) peroxidase-conjugated swine anti-rabbit (1 : 60; DAKO). The reaction was developed with diaminobenzidine–hydrogen peroxide and counterstained with Mayer's haemalum. The monoclonal antibodies used were (dilution specificity): leu 4 (1 : 200 T lymphocytes); leu 3a (1 : 100 helper/inducer T cells; leu 2a (1 : 200 suppressor/cytotoxic T cells); M718 (1 : 50 monocytes/macrophages); leu M1 (1 : 20 monocytes); OKT6 (1 : 200 Langerhans' cells); leu 7 and leu 11b (both 1 : 10 K cells/NK cells/large granular lymphocytes); leu 14 (1 : 50 B cells).

The presence of positive cells in each section, both at the dermo-epidermal interface and perivascularly, was scored on a semiquantitative scale (10) (see Table I). From every patient, sections from normal skin, stained with OKT6 (CD1) and M718, served as control for CD1/M718 activity in abnormal skin. Histology (of the cryosections) showed varying degrees of basal cell degeneration in all LE-patients and spongiosis in most patients with polymorphic light eruption.

Infiltrates in and around pilosebaceous units could not be evaluated, since adequate amounts of hair follicles were present in only 5 biopsies.

Deposits of immunoreactants. Three-mm punch biopsies from 46 different phototest sites were performed for immunofluorescence (IF) microscopy (39 biopsies from 16 LE patients and 7 from 6 patients with polymorphic light eruption). Simultaneously, clinically normal skin at a corresponding site was biopsied. From all LE-patients a biopsy from a spontaneously evolved lesion had been tested before (see Table II). Biopsies were taken 7–10 days after onset of the test-exposures. In 8 patients, consecutive biopsies were also taken 4–6 weeks after the exposures. Skin sections were tested for the presence of immunoglobulin classes IgA, IgG, IgM and C3c at the basal membrane zone (BMZ), using commercially available FITC-conjugated antisera (DAKO, Copenhagen) and a trans-illumination microscope (Zeiss) (see for a detailed description of the technique used: Velthuis et al. (11)). Granular deposits comprising more than 10% of the BMZ length were considered positive. When deposits extended for more than 75% along the BMZ the term 'bandlike staining' (lupus band) was used.

Both in sections for IF microscopy and in infiltrate analysis the histological presence of basal cell degeneration and the presence or absence of dermo-epidermal infiltrate (interface dermatitis), or an infiltrate in the stratum papillare were recorded. Significant associations between these histologic features and immunohistopathology were tested by Fisher's exact test.

RESULTS

Infiltrate analysis.

In both polymorphic light eruption and LE, *dermal infiltrates* (Table I) consisted mainly of CD3+, CD4+, and CD8+ cells. The CD4/CD8 ratios in different biopsies ranged from 1 : 1 to 6 : 1. CD15+, leu 7+, CD22+ cells were sparse, whereas CD16+ cells were never seen. The amount of CD1+ cells in the dermis was significantly greater in polymorphic light eruption (median: 26–50%) than in LE (1–5%) (Fig. 1), although in the former the range was wide (1–75%). The LE patients did not show clear differences between the percentages of the various cell types in the two locations (perivascular or at the interface), but did so for the dominance of M718+ cells at the interface.

In the *epidermis* of both disorders the amount of CD1+ cells had diminished as compared with non-irradiated skin. In 8 biopsies (6 from LE patients and 2 from patients with polymorphic light eruption), these cells were completely absent. In both LE and polymorphic light eruption, other cell types were scarce in numbers, never exceeding 15 cells per section. In polymorphic light eruption, 4 biopsies showed CD3+ cells, in one case associated with CD8+ and CD4+ phenotypes. In LE (Table III) CD3+ cells were invariably found, frequently in combination with CD4+, CD8+, M718+ (Fig. 2) and CD15+ cells. M718+ and CD15+ cells were

Table II. *Immunofluorescence findings in the basal membrane zone in skin biopsies of patients with lupus erythematosus*

	Non-induced		Induced			
Patient	Lesion	Normal	UVA (7–10 days)	UVB (7–10 weeks)	UVA (4–6 weeks)	UVB (4–6 weeks)
1	AGMC	–	M/gc	MC/g	AGMC	MC/ag
2	AGMC	–	MC/g	M/c	MC/g	M/agc
3[a]	AGMC	–	gc	G*M		C
4	AGC	–	gmc	–		M
5	AGMC	a	agm	AM/gc	A	AGC
6	MC	M	M	M		
7[a]	AGMC	G*	–	A*G*MC		
8	–	–	–			
9	AGMC	–	–	M/ac		
10	G*M	G*	G*MC/a	G*MC		G*/mc
11	MC	–	–	C		G*/mc
12	MC	–		MC		G*
13	–	–	–	C		
14	–	–	–			
15	AGMC	–	–	–		
16	GMC/a	agm		GC		

A=IgA, G=IgG, M=IgM, C=C3, capitals=bandlike deposits (>75% of BMZ length), lower case letters=patchy deposits (<75%, >10% of BMZ length), *=dustlike particles, blank=not tested, –=negative, diagnoses in patients 1–6: DLE / 7–14: SCLE / 15, 16: SLE.

[a] =Skin signs of both DLE and SCLE.

sometimes seen in clusters at sites of extensive epidermal necrosis.

Deposits of immunoreactants

Results of the LE patients are summarized in Table II. Early biopsies showed immunoreactants (others than those deposited in the clinically normal skin) in 12 of the 16 patients tested. In 10 of them, narrow bandlike staining developed, consisting primarily of IgM and C3c. In one patient only discontinuous deposits were observed; and in another no conclusions could be drawn as the healthy skin also showed deposits of three types of immunoglobulins. There was no association between the presence of band-like BMZ staining and the extent of basal cell degeneration or of interface dermatitis or upper dermal infiltration.

Another pattern, described previously by Nieboer and others (12) as dust-like particles (DLP) was found in 5 patients (10 biopsies) (Table II), representing a distinct pattern (Fig. 3) of very fine IgG granules scattered throughout the cytoplasm of the epidermal cells, the region directly subepidermal and the dermal infiltrates; epidermal nuclear staining is not very prominent. Although not significantly associated, this finding was found exclusively at sites of extensive basal cell degeneration in irradiated skin; in 2 of these patients with extensive but focal basal cell degeneration, the DLP-pattern was only seen in and around

Table III. *Phenotypes of infiltrative cells in the epidermis or with epidermal contact in 17 biopsies from UV-provoked LE-lesions*

	Number of positive cells		
Phenotypes	<5	⩾5	Total
leu 4 (CD3)	3	14	17
leu 3a (CD4)	9	6	15
leu 2a (CD8)	11	3	14
M7 18	8	5[a]	13
leu M1 (CD15)	7	2[a]	9
leu 7	0	0	0
leu 11b (CD16)	0	0	0
leu 14 (CD22)	0	0	0

[a] Clusters of leu M1+ cells in one patient, clusters of M7 18+ cells in four.

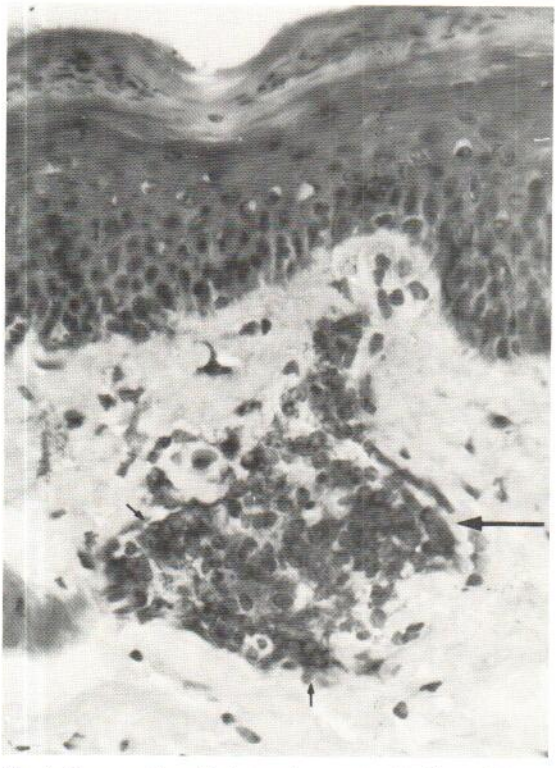

Fig. 1. Large and small clusters (*arrows*) and solitary CD1+ (OKT6) cells in a dermal infiltrate of an induced lesion in polymorphic light eruption.

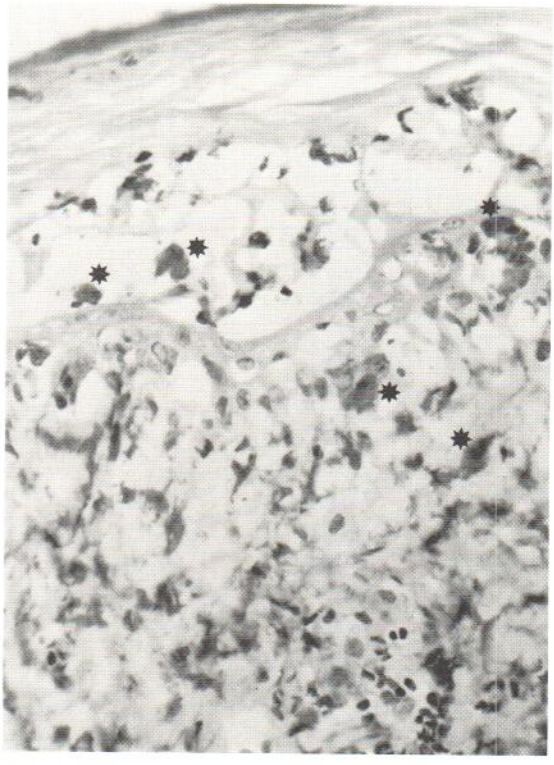

Fig. 2. M718+ cells at site of extensive epidermal necrosis in induced LE lesion. (Some clearly positive cells are marked with an asterisk.)

these areas. In the polymorphic light eruption group, 2 patients showed discontinuous granular deposits in healthy skin (one IgM, the other C3c). A third patient showed development of discontinuous C3 deposits at the BMZ after UVB irradiation.

DISCUSSION

There is a great similarity between the phenotypes of the inflammatory cells in spontaneously evolved LE lesions and those induced by our phototest irradiations. There is generally little epidermal infiltration in spontaneously evolved LE lesions, but if present it shows CD4 and CD8 phenotypes (10, 13, 14). We have frequently found epidermal infiltration in induced lesions and a greater variety of phenotypes, most frequently CD3+, CD4+ and CD8+ cells. In the dermal infiltrates of both types of lesions, CD3+, CD4+, and less frequent CD8+ cells are visible; there are few CD1+ cells, almost no CD22+ cells (10, 13–15) and a relatively large quantity of cells with a macrophage-phenotype (15).

The main difference between the inflammatory infiltrates in LE and polymorphic light eruption is the relatively large quantity of dermal CD1+ cells in polymorphic light eruption. This has also been report-

Fig. 3. Dust-like particles of IgG: in epidermis, direct subepidermal (*arrows*) and in infiltrates (*asterisk*). *ED*, epidermis; *D*, dermis.

ed in spontaneously evolved lesions from polymorphic light eruption (16) and in positive patch tests (17). These cells are assumed to represent antigen-presenting cells migrating from epidermis to lymph nodes to elicit a type IV allergic reaction. As in our study, few CD1+ cells were seen in LE, such a mechanism appears non-operative in UV-induced LE.

Based on recent experiments, the theory was formed that an antibody dependent cellular cytotoxicity (ADCC) reaction might be responsible for basal cell degeneration following UV irradiation (18). TNP (trinitrophenol)-sensitized keratinocytes of several species showed a high susceptibility to ADCC, mediated by lymphocytes (CD4+ cells) and monocytes (CD11+ cells), whereas eosinophils and neutrophils were ineffective in this respect (19). In our study, CD4+ cells and cells staining with the two monocyte markers, CD15 and M718, were frequently found in contact with epidermal cells. Significantly more M718+ cells were found at the interface and in the epidermis than in the perivascular infiltrate, suggesting an active migration towards the epidermis. From the preponderance of M718+ cells over CD15+ cells, both in dermal infiltrates and in the epidermis, one may assume that, in addition to monocytes, skin macrophages are also present. In contradiction to an ADCC-type of reaction is the almost complete absence of specific killer cell phenotypes, especially the Fcγ receptor (CD16).

Alternatively, light-induced skin lesions in LE may result from an immune-complex mediated injury. Both spontaneous and radiation-induced LE-skin lesions nearly always show granular or homogeneous deposits of immunoglobulins and complement factors in the basal membrane zone (20). The early occurrence after UV-inducement of IgM and C3 in 11 out of 15 patients supports this theory. However, since immunoreactants are also found in clinically normal skin of SLE patients (in approximately 70% (20)) other factors leading to injury must also be involved. In addition, the murine type lupus, seen in NZB/NZW F_1 mice does not include skin lesions despite the presence of immunoreactants at the BMZ (21).

In spontaneously evolved lesions in polymorphic light eruption, no or only weak homogeneous staining of immunoglobulin at the basal membrane zone (BMZ) has been noted (22). Discontinuous IgM and C3 deposits, as found in this study, have also been seen in normal skin of healthy individuals (23, 24) and probably have no diagnostic or specific pathologic implications.

In conclusion: we have demonstrated that in artificially irradiated skin of patients with LE, immunohistochemical changes can be induced similar to those in spontaneously evolved lesions, but distinct from those in artificially irradiated polymorphic light eruption. Although the results do not provide conclusive arguments for either of the two prevailing theories on the pathogenesis of light-induced LE lesions, they underline the important role of light in the pathogenesis of LE.

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