

DRUG-INDUCED LUPUS ERYTHEMATOSUS SYNDROME

Immuno-pathological, Electronmicroscopical and Serological Studies

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Abstract. Skin biopsies from three patients suffering from lupus erythematosus syndrome induced by hydralazine and procainamide were studied by immunofluorescence microscopy and electron microscopy. Deposits of IgM were found in all, IgG in one and complement C3 in two of the patients in the dermo-epidermal junction as seen in "spontaneous" systemic lupus erythematosus (SLE). The deposits disappeared when the clinical symptoms subsided. Electron microscopy showed no intracytoplasmic inclusions in the endothelial cells of the dermal vessels as typically seen in spontaneous SLE. Two of the patients had very high serum titres of granulocyte-specific antinuclear factors (GS-ANF) of the IgG class in the acute phase. They disappeared rapidly when the clinical symptoms subsided. In contrast, the essentially lower titres of IgG organ-nonspecific antinuclear factors (ON-ANF) persisted for long periods.

The administration of certain drugs, e.g. procainamide and hydralazine, may give rise to production of antinuclear factors (ANF) and LE cell phenomena. In some of the patients receiving these drugs, clinical symptoms suggestive of systemic lupus erythematosus (SLE) appear, such as fever, joint pains and occasionally skin eruptions. The serological and clinical manifestations usually disappear after withdrawal of the drug (1, 7, 14, 19).

In the skin of patients with spontaneous SLE, deposits of immunoglobulins and complement are regularly demonstrated in the dermo-epidermal junction, and virus-like intracytoplasmic inclusions are almost invariably found in the vascular endothelial cells. Such deposits and inclusions have been detected in clinically involved as well as in uninvolved skin (4, 5, 6, 10, 11, 12, 13, 15, 17).

Three patients with drug-induced SLE are presented. In all of them, skin biopsies were examined, by direct immunofluorescence microscopy, for immunoglobulin (Ig) and complement deposits. In two of them, virus-like inclusions were sought by electron

microscopy. The sera of 2 patients were studied for granulocyte-specific and organ-nonspecific antinuclear factors (GS- and ON-ANF) by titration, and all blood samples were investigated for LE cells.

MATERIAL AND METHODS

Case Reports

Patient 1, a 49-year-old woman, was admitted because of palpitations due to multiple extrasystoles. Thyrogenic myxoedema was diagnosed, and her serum contained microsomal thyroid antibodies. There were no joint pains, and tests for ANF and LE cells were negative. Treatment was started using procainamide 1.5 g and L-thyroxin 0.15 mg daily. After 4 months she developed severe pain in several joints including those of the fingers, but no joint swelling was noticed. ESR had increased to 40 mm/h and the serum concentration of IgG was raised (16.7 g/l), while the serum contained normal amounts of complement C3 and C4. The LE cell test was strongly positive. Urinalysis and kidney function were normal, and she had no skin rash. She was found to have achlorhydria, a low serum-B₁₂ level and a pathological Schilling test as well as parietal cell antibodies in her serum, while the bone marrow was normoblastic. Procainamide was withdrawn and the joint pains subsided gradually over 6 weeks; the ESR and serum IgG became normal. One year later the LE cell test was borderline positive.

Patient 2, a 37-year-old man, had severe hypertension (blood pressure 250/150 mmHg) and bilateral papillary oedema. He had a normal ESR, normal kidney function, minimal proteinuria and a normal urine sediment. The blood pressure did not respond to large doses of guanethidine and methyldopa, and one year later hydralazine was added to the regimen. The blood pressure came down, and he was maintained on guanethidine 20 mg, methyldopa 2 g, and hydralazine 400 mg per day for 5 years. He was found to be uremic with a serum creatinine of 10 mg per 100 ml, the blood pressure was again 250/150 mmHg, and he had bilateral papillary oedema. Routine analysis for ANF revealed a strong reaction, while LE cell tests were negative. The serum was not studied in detail for ANF by titration. He was not complaining of joint pains, and he presented no signs of arthritis or dermatitis. Serum electrophoresis was

normal, and he had normal concentrations of complement C3 and C4. He died suddenly with signs of a cerebral hemorrhage. No autopsy was performed.

Patient 3, previously published (3), was a 30-year-old male with a 10-year history of generalized scleroderma (acrosclerosis). With the rationale that hydralazine inhibits collagen biosynthesis (2), he was tentatively treated with hydralazine 75 mg daily. After about one year of treatment he started suffering from anorexia, fatigue, joint pains, productive cough, diarrhoea and fever about 39–40°C. He had skin petechiae, an erythematous facial rash, anemia, granulocytopenia, increased serum IgG and IgM, a positive Wassermann reaction and a positive LE cell test. There was erythrocyturia and proteinuria (2 g/day), while serum creatinine and the complement values were normal. He was treated with betamethasone for about 3 months. The temperature became normal within a few days, and he returned to his original sclerodermic state. During the following months most laboratory tests returned to normal, but the LE cell test was still positive 8 months later.

Skin biopsies

Skin biopsies were obtained in the period when clinical evidence of drug-induced SLE was present, and were repeated in patients 1 and 3 when all symptoms had disappeared, i.e. 4 and 11 months after withdrawal of the offending drugs. The biopsies were obtained from clinically normal skin in patients 1 and 2 and from sclerodermic skin in patient 3. The second biopsies were taken from skin adjacent to previous biopsy sites. The initial biopsies from patients 1 and 3 were divided in two parts, one for immunohistochemical staining and one for electron microscopy. Patient 3 also had a biopsy performed for electron microscopy before hydralazine treatment was started.

Immunohistochemical staining

The biopsies were immediately frozen in liquid nitrogen, and 4–8 µm thick sections were cut in a cryostat. The sections were air-dried for 15 min, washed in saline for 30 min and incubated with one drop of diluted conjugate in a moist chamber for 30 min. Blocking with unconjugated antisera was included in each experiment.

Fluorescein isothiocyanate labelled rabbit IgG specific for human γ , μ and α chains and the β 2c component of human C3 (Dako, Copenhagen) was used after appropriate specificity tests on monoclonal bone marrow specimens from patients with myelomas and macroglobulinaemia as described earlier (20). All conjugates showed monospecific reactions in crossed immunoelectrophoresis (20), and the F:P ratios as estimated by OD 495/280 nm were close to 0.6. No non-specific staining was seen at the working dilution.

The preparations were examined in a Leitz Ortholux fluorescence microscope equipped for transmittent light illumination using an Osram HBO 200 mercury lamp as light source, a KP 490 nm interference filter for primary light selection, a Tiyoda wide-angle dark-field condensor and a 3 mm OG 530 nm glass filter as barrier filter.

Serological technique

Sera from patients 1 and 3 were investigated for the occurrence and titres of IgG and complement-fixing GS-ANF and ON-ANF as described earlier (20). Human thyroid

Table I. Deposits of immunoglobulins and complement C3 in the dermo-epidermal junction of the skin of 3 patients with drug-induced lupus erythematosus syndrome

Patient	Drug	Initial biopsy	Second biopsy
1	Procainamide	IgG, IgM and C3	Negative
2	Hydralazine	IgM	Not done
3	Hydralazine	IgM, C3	Negative

cryostat sections and smears of isolated and washed human leucocytes served as nuclear substrates. The results were read in a Leitz Orthoplan fluorescence microscope equipped for incident light illumination as described previously (20). At the chosen working dilution (1:25) no non-specific staining could be found. Sera and conjugates were diluted in phosphate-buffered saline, pH 7.2.

Electron microscopy

The specimens were fixed in 6% glutaraldehyde in Veronal acetate buffer, pH 7.2, with 7.5% sucrose. After osmification the specimens were washed, dehydrated in a series of alcohols of increasing concentration, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Siemens electron microscope (Elmiskop 1A) at 80 kV with a double condensor system.

RESULTS

Immunohistochemical studies

In all three biopsies obtained during the active phase of the SLE syndrome, deposits of Ig were found in the dermo-epidermal junction (Table I). IgM was found in all three biopsies (Fig. 1), while IgG was found only in the biopsy from patient 1 (Fig. 2). Complement C3 was demonstrated in the biopsies



Fig. 1. Deposits of IgM in the dermo-epidermal junction of the skin. Patient 2.

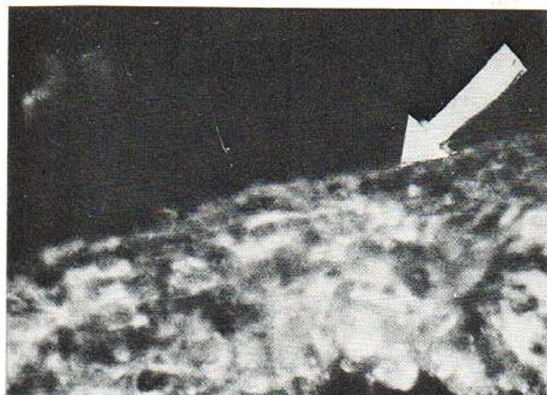


Fig. 2. Deposits of IgG in the dermo-epidermal junction of the skin. Patient 1.

from patients 1 and 3. The deposits formed a narrow homogeneous band. Following blocking procedures no fluorescence was seen. No deposits could be demonstrated in the biopsies from patients 1 and 3 after the symptoms had subsided.

Electron microscopy

No intracytoplasmic inclusions were found in any of the biopsies examined. The dermo-epidermal junction, vascular endothelium and dermal connective tissue revealed no pathological ultrastructures. In none of the biopsies could cell infiltrates be demonstrated in the dermis.

Serological studies

The titres of IgG GS-ANF and ON-ANF found in the serum of patients 1 and 3 at the various stages of the SLE syndrome are recorded in Table II. Both patients had high titres of IgG GS-ANF and low titres of IgG ON-ANF in the first serum specimen. Both had complement-fixing ON-ANF. On withdrawal of procainamide and hydralazine, the titres of IgG GS-ANF fell to unmeasurable levels. In contrast, for almost 3 months, the ON-ANF levels remained essentially unchanged in patient 1 and somewhat increased in patient 3 during the next 6 weeks. After 10 months, patient 1 no longer had complement-fixing ANF corresponding to the absence of classical LE cell phenomena. Patient 3 still had a positive LE cell test after 4 months, corresponding to essentially unchanged titres of complement-fixing ON-ANF.

DISCUSSION

Previously, deposits of Ig and complement in the dermo-epidermal junction have been found in in-

Table II. Occurrence and titres of IgG and complement-fixing (CF) GS-ANF and ON-ANF in sera from 2 patients with drug-induced lupus erythematosus syndrome at different stages of the disease

Patient	Time of study (days after drug withdrawal)	IgG GS-ANF titre	IgG ON-ANF titre	CF ON-ANF titre
1	Active phase	4 096	512	16
	23		512	16
	80		512	8
	140		128	2
	270		128	0
	365		128	0
	435		64	0
3	Active phase	4 096	128	128
	20	1 024	128	64
	45		512	64
	115		512	64

involved as well as uninvolved skin of patients with spontaneous SLE (4, 5, 6, 11, 15, 17). The deposits may represent antigen-antibody complexes or anti-basal lamina antibodies (19). In a previous paper, Burnham et al. (6) found no deposits of Ig and complement in the dermo-epidermal junction of 2 patients with drug-induced SLE. However, in the present study, Ig deposits were demonstrated in all 3 patients with drug-induced SLE.

The serum of patient 3 contained a factor (antibody?) which precipitated complexed hydralazine-deoxyribonucleic acid (3).

Intracytoplasmic inclusions in vascular endothelial cells demonstrated by electron microscopy are a constant finding in involved as well as uninvolved skin of patients with spontaneous SLE (10, 12, 13). In scleroderma, intracytoplasmic inclusions are occasionally found in endothelial cells of the dermal vessels (10). Györkey et al. (9) reported on electron microscopic studies of kidney biopsies from 2 patients with procainamide-induced SLE in whom no viral inclusions were found. The same authors found inclusions in all of 52 kidney biopsies from patients with spontaneous SLE. The present findings suggest that skin from patients with drug-induced SLE resemble spontaneous SLE in the occurrence of deposits of IgG and complement in the skin, but differ in the absence of virus-like structures.

In patients with spontaneous SLE, IgG ON-ANF mostly occurs in high titres (16). In active phases of lupus nephritis the titres of complement-fixing ON-ANF are also high, whereas remissions are regularly accompanied by a fall in ON-ANF possessing complement-fixing properties (18). GS-ANF have been found in patients with spontaneous SLE, but in most cases they are hidden by high titres of ON-ANF and can only be demonstrated after absorption of the latter (8).

The occurrence of GS-ANF in drug-induced SLE has not been published before. The most interesting observation would be the rather close coherence between clinical disease activity and the presence of GS-ANF and the subsequent disappearance of both on discontinuation of drug treatment. GS-ANF have been shown to participate in immune complexes in sera and synovial fluids of patients with rheumatoid arthritis (21, 22). It seems reasonable to suggest that they may form complexes in drug-induced SLE as well, and thus participate in complex deposition in the tissues including the skin.

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