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IMMUNOFLUORESCENT AND ELECTRON-MICROSCOPE FINDINGS IN THE UNINVOLVED SKIN OF PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Abstract. Specimens of uninvolved skin of 20 patients with systemic lupus erythematosus (SLE) were studied using a direct immunofluorescent technique (IF) and the electron microscope (EM). EM examination revealed deposits of electron-dense material below the basal membrane, among collagen fibers in the dermis and in the walls of dermal blood vessels. The direct IF technique revealed granular "band" deposition of immunoglobulins and complement at the dermo-epidermal junction as well as granular deposition in the walls of blood vessels. There was close correlation between the IF and EM findings. It was concluded that the EM is of particular value in precisely delineating the location and ultrastructural pattern of skin deposits. The IF technique is not limited in relation to the size of area which can be studied and makes it possible to identify the specific immune fractions present. It is suggested that these two methods are mutually complementary in providing information concerning the deposition of immunoglobulins taking place in the uninvolved skin of SLE patients.

Key words: Systemic lupus erythematosus; Immunofluorescence; Electron microscope; Uninvolved skin

It has been demonstrated that in systemic lupus erythematosus (SLE), immune complexes are deposited in the blood vessels of various organs (31, 40) and also in the skin, which has been found to be the most frequently involved organ (25). It has further been shown that the deposition of immune complexes in clinically uninvolved skin at the dermo-epidermal (D–E) junction is characteristic of and diagnostic for SLE (4, 5, 16).

It was the aim of the present study to examine the immune deposits in uninvolved skin of SLE patients by using both a direct immunofluorescent (IF) technique and the electron microscope (EM) in order to evaluate the two methods.

MATERIALS AND METHODS

The patient material comprised 20 patients (19 females, one male), with an age range from 20 to 66 years who had been diagnosed as suffering from SLE on the basis of the criteria established by the American Rheumatism Association (6), and who were under observation in an outpatient department. Among these, 11 patients were receiving systemic steroids and 3 received combined therapy with Imuran and steroids. Impaired renal function was found in 5 patients, in 3 of whom renal biopsy was performed with the following findings: mesangial lupus nephritis in one; diffuse proliferative glomerulonephritis in one; and membrano-proliferative glomerulonephritis in one. Skin biopsy samples were obtained from a clinically uninvolved area located in the distal extensor part of the arm, by using a 4–5 mm skin punch, with local anesthesia effectuated by a 2% esracaine solution. One-half of the biopsy specimen obtained was studied using immunofluorescent (IF) technique and the other by electron microscope (EM).

Direct IF examination

The specimens intended for study by IF technique were immediately frozen with "Cryokwik" (fluorinated hydrocarbons) and stored at −90°C until used. Sections 4 μm thick were cut on an I.E.S. microtome at −24°C. Prior to staining with fluorescein-labelled antibody reagents the sections were washed in phosphate-buffered saline at pH 7.2 to remove any unbound globulins. A drop of the appropriate fluorescent-labelled antibody reagent was placed on the sections, which were then incubated in a moist chamber at room temperature for 30 min. The sections were washed and mounted under a glass cover-slip with buffered 10% glycerol. The fluorescent-labelled antisera used (obtained from Hyland Laboratories, California) included monospecific antisera to human IgG, IgM, IgA, C_3' and fibrinogen. Their fluorescein/protein ratios and specific antibody concentrations are listed in Table I. The conjugates were diluted as follows: IgG, 1:20; $\beta_1c\beta_1a$, 1:20; IgA, 1:20; IgM, 1:15; fibrinogen, 1:20. These dilutions were found optimal for minimized nonspecific staining. IF controls consisted of sections treated with unlabelled antisera and then with the respective label-

Table I. *Fluorescein/protein ratios and antibody concentrations of anti-human antisera*

	IgG[a]	IgM[a]	IgA[a]	$\beta_1c\beta_1a$[d]	Fibrinogen
Fluorescein/protein ratio					
Weight ratio[b]	7.4	6.4	8.0	8.1	Unspecified
Molar ratio	3.0	2.6	3.3	3.3	Unspecified
Specific antibody concentration[c]	1.2	2.3	2.2	2.0	Unspecified

[a] Goat origin.
[b] Measured as μg/mg.
[c] Measured as mg/ml.
[d] Rabbit origin, lot no. 2108 M002A_1.

led antibody to demonstrate specific inhibition. Fluorescence of immune deposits (not including a finding of fibrinogen alone) was expressed in three degrees of strength from + to +++.

EM examination

The skin specimens were double-fixed in 2% glutaraldehyde and 1% osmium tetroxide, dehydrated and embedded in flat Epon blocks. Sections 1 μm thick were stained with toluidine blue for screening. After selection of appropriate areas under the light microscope, ultra-thin sections were cut, double-stained with uranyl acetate and lead citrate, and examined with a Philips 300 electron microscope. In every case several sections of each of at least five blocks were studied. The amount of deposit was graded as minimal (+), moderate (++) or massive (+++), minimal being defined as 1 or 2 minute deposits appearing in only 1 or 2 blocks, moderate as several small deposits appearing in all sections below the epidermis and sometimes among the dermal collagen fibers, and massive as numerous small and large deposits appearing below the epidermis and among dermal collagen fibers.

RESULTS

Direct IF examination

Immune deposits were found in two locations: (*a*) "band" deposition at the D–E junction in 18 patients (90%) (Fig. 1), and (*b*) deposition in the walls of the dermal blood vessels in 3 patients (15%). In the latter 3 cases there were immune deposits at both sites. In 2 patients no immune deposits were seen. In all cases immune deposits had a granular pattern. In 15 patients the fluorescence at the D–E junction was strong (++ or +++) and in 3 patients it was weak (+) (Table II). IgG showed the strongest fluorescence and was also the immunoglobulin most commonly found at the D–E junction, whereas IgA was the least commonly found immunoglobulin. The distribution of immune deposits found at the D–E junction is illustrated in Table III. Seven patients had immunoglobulins only; 4 patients had immunoglobulins + C'_3; 5 patients had immunoglobulins + fibrinogen; and 2 patients had immunoglobulins + C'_3 + fibrinogen. The distribution of immune deposits found in walls of dermal blood vessels (3 patients) is illustrated in Table IV. One patient had immunoglobulins alone and 2 patients had immunoglobulins + fibrinogen.

EM examination

In all specimens examined the basal membrane (BM) was seen to be thickened at the D–E junction. In 17 patients (85%) there were extracellular deposits of electron-dense material (EDM) under the BM with occasional downward infiltration among the collagen fibers of the dermis. At the BM itself,

Table II. *Immune deposits in uninvolved skin of 20 SLE patients as determined by IF and EM examination*

Patients	IF D–E junction	EM Below B.M. and among collagen fibers
1	++	++
2	++	+
3	++	++
4	++	++
5	++	+
6	+	++
7	–	–
8	+++	+++
9	+	++
10	+	–
11	+++	++
12	++	++
13	+++	++
14	++	++
15	+++	+++
16	++	+
17	++	+
18	+++	++
19	–	–
20	+++	+++
Total positive	18/20 (90%)	17/20 (85%)

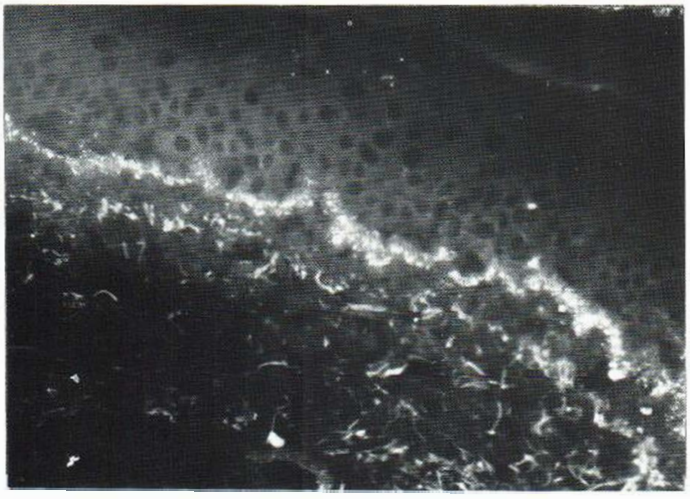

Fig. 1. Granular deposition of $\beta_1c\beta_1a$ at the D–E junction of uninvolved skin. ×160.

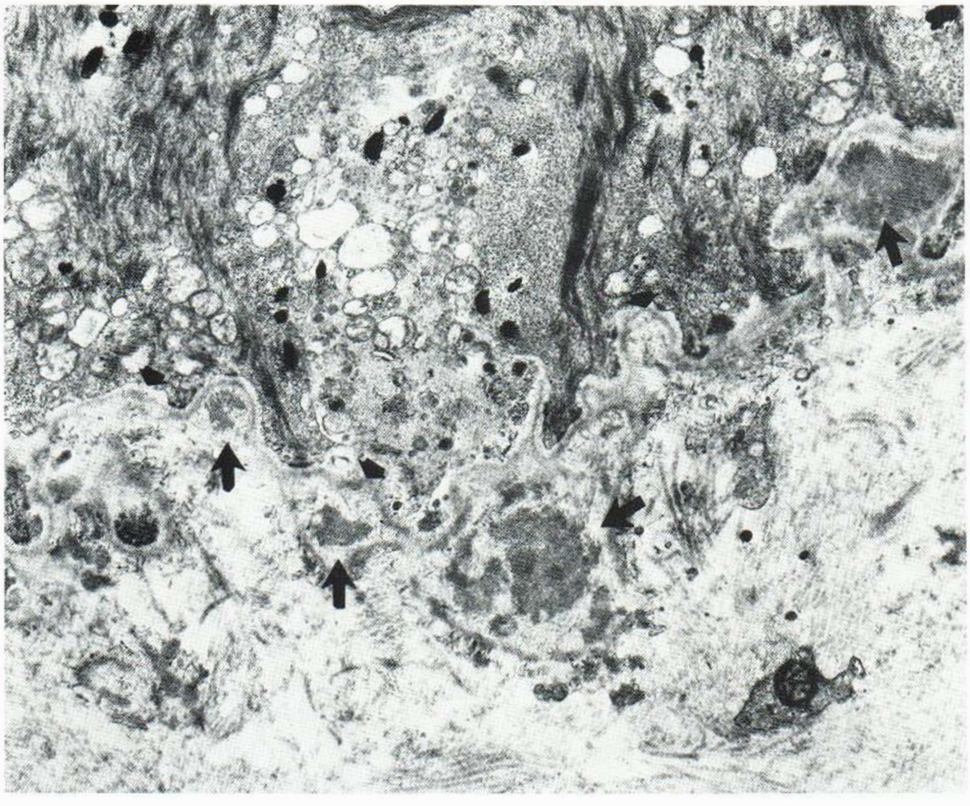

Fig. 2. Multiple deposits of EDM below the basal membrane (*large arrows*). The basal membrane is thickened (*small arrows*). ×13600.

Table III. *Immune deposits at the D–E junction*

Patient	IgG	IgM	IgA	$\beta_1C\beta_1a$	Fibrinogen
1	×	×			
2	×				
3	×	×		×	
4	×	×	×	×	
5	×	×			
6	×	×		×	
7					
8[a]	×		×	×	×
9	×	×			
10	×				
11	×			×	
12	×				
13	×				×
14	×				×
15[a]	×	×	×		×
16		×	×		×
17		×			×
18[a]	×	×	×		
19					
20	×	×		×	×
Total	17	11	5	6	7

[a] Immune deposits present also in walls of dermal blood vessels.

however, no deposits were seen (Fig. 2). In 3 patients the deposits were massive (+++), in 10, moderate (++) and in 4, minimal (+) (Table II). These sub-epidermal deposits were seen as separate, irregular masses of varying size rather than as band-like formations. In 3 patients no EDM was found below the BM; in 2 of these the IF examination was also negative and in one, minute amounts of IgG were seen deposited at the D–E junction. EDM was found in the walls of dermal blood vessels in only 3 patients, all of whom showed EDM below the BM and among the dermal collagen fibers. It is of note, however, that of these 3 patients, in only one had the IF technique shown deposits in the walls of the blood vessels.

In the walls of the blood vessels, EDM was not seen arranged in a complete circle but as separate masses. In 2 patients "organized" deposits were found, seen as a symmetrical arrangement of parallel bands appearing periodically in a straight or concentric pattern of alternately dark and light areas (Fig. 3). In 4 patients within the cytoplasm of the endothelial cells of capillaries and arterioles there were tubular structures, 200–250 Å in diameter which resembled "myxo-virus like particles" (intracytoplasmic microtubular inclusions) (Fig. 4).

Table IV. *Immune deposits in walls of dermal blood vessels*

Patient	IgG	IgM	IgA	$\beta_1C\beta_1a$	Fibrinogen
8	×	×			×
15	×	×	×		×
18	×	×			
Total	3	3	1	0	2

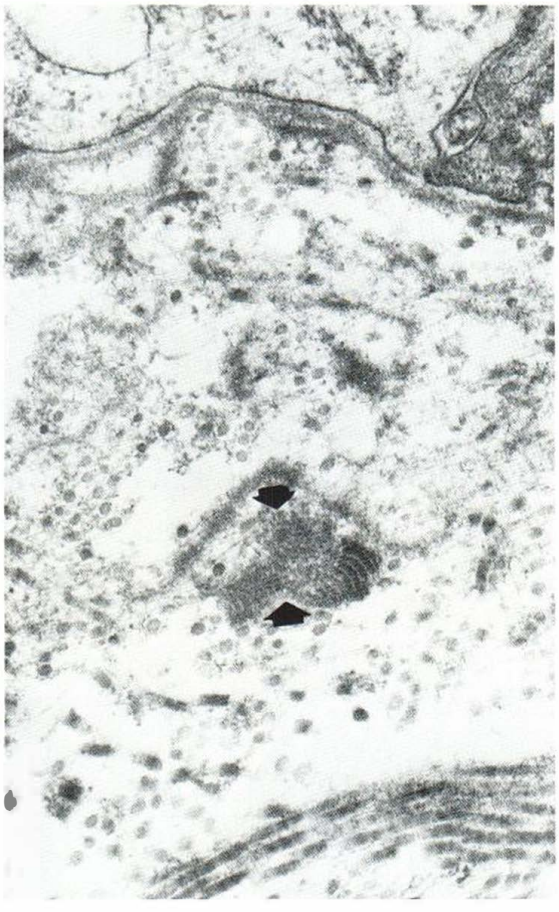

Fig. 3. "Finger-print" organized deposits in the upper dermis (*arrows*). ×26 000.

DISCUSSION

Studies of skin taken from patients with SLE have shown immunoglobulins and complement to be present in three different areas: as a "band" deposition at the D–E junction of both involved and uninvolved skin (lupus band test) (2, 4, 7, 32, 33, 36, 37),

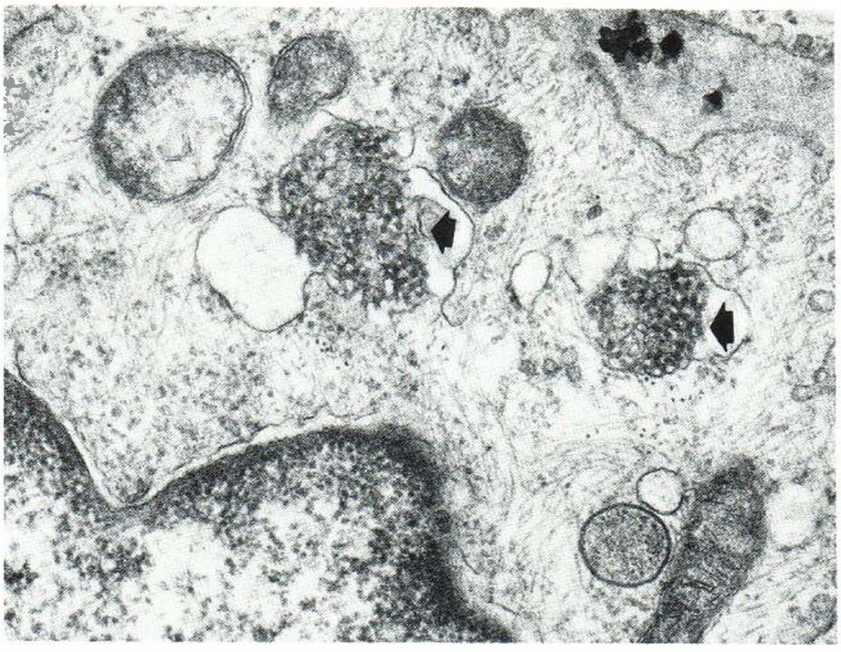

Fig. 4. Two aggregates of "myxovirus-like particles" within the cytoplasm of an endothelial cell (*arrows*) ×40 000.

in the walls of blood vessels (9), and in the connective tissue of sub-epidermal papillae (39). A positive lupus band test (LBT) in uninvolved skin has come to be considered specific and diagnostic for SLE (4, 5, 16), having been found in 50–60% (32, 37, 38) of SLE patients, but immune deposits in the walls of blood vessels and in the sub-epidermal papillae are less specific for SLE (9, 39). EM studies performed by Grishman & Churg (15) showed EDM in the uninvolved skin of SLE patients below the BM, among collagen fibers, and in the walls of blood vessels; the EDM was considered to resemble the wire-loop lesions present in glomeruli and extraglomerular blood vessels (11, 13, 22). Lambert et al. (24) carried out a comparative study of the histoimmunological and ultra-structural findings of healthy and pathological skin in lupus erythematosus, but their findings were inconclusive.

It was the aim of this study to compare the findings obtained using IF and EM for the examination of uninvolved skin of SLE patients, thereby evaluating the accuracy, advantages and drawbacks of each method. Whereas IF technique revealed immune deposits in two main locations (D–E junction and walls of blood vessels), the EM examination showed such deposits to be present in three different areas (below the BM, among collagen fibers of the dermis, in walls of blood vessels). The presence of EDM below the BM and among the collagen fibers of the dermis (in 17 of 20 patients) was, however, closely correlated with the "band" deposition at the D–E junction detected by IF examination (18 of 20 patients).

EM examination made it possible to identify even minute deposits and also to determine with precision their location and ultrastructural pattern. Deposits at the D–E junction which by IF technique appeared as a continuous granular band, were seen by EM examination to be separate masses varying in size. EM examination was able to establish that no deposits were located in the BM itself, a fact which could not be demonstrated by the IF technique. With the IF technique, in contrast, it was possible to identify the various immune fractions deposited in the skin, which included IgG, IgM, IgA, fibrinogen and C_3'. In both the IF and EM examinations deposits were much less commonly found in the walls of blood vessels than at the other sites, as has been reported by other authors as well (3, 8, 21, 36). The IF was positive in the walls of blood vessels only in patients with a positive LBT. There was no correlation between the IF and EM findings as concerns deposits in the blood vessel walls, possibly due to the limited amount of tissue which can be studied by the EM as compared with that which can be studied by the IF technique.

"Organized deposits", detected by us in the skin of only 2 patients, were regarded by Grishman (15) as a quite characteristic finding in SLE, having been observed in involved and uninvolved skin (15), kidney (10, 12) heart, spleen, and joint capsule (14).

Intra-cytoplasmic microtubular inclusions (ICMI), found by us in 4 patients, are not specific for SLE and have been demonstrated in the affected tissues of other pathological processes, particularly collagen diseases, including dermatomyositis (20, 26, 30), discoid LE (19), progressive systemic sclerosis, Sjögren syndrome (26, 35) and epithelial tumors (27). In SLE they have been demonstrated to be present in involved and uninvolved skin (17, 23, 28, 29) and in renal lesions (18, 26). A rise in the incidence of ICMI was observed in SLE patients showing activation of the disease and after ultraviolet irradiation of the skin (1). In this respect it is of interest that of the 4 patients in whom ICMI were found in our study, 2 showed disease activation. Despite the resemblance of ICMI to the nucleo-capside of para-myxovirus, most investigators suggest that ICMI represents a cellular reaction production rather than being the pathogenetic factor itself (1, 34).

On the basis of this study, it is suggested that the combined use of both immunofluorescent and electron-microscope techniques can be valuable not only as a diagnostic test but also in helping us to clarify the pathological processes taking place in SLE.

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