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IMMUNOFLUORESCENCE STUDY OF CUTANEOUS BLOOD VESSELS OF 16 PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Abstract. In 16 patients with systemic lupus erythematosus, blood vessels in the affected region of the skin were examined regarding localization of immune complexes. Deposition of IgG, IgA, IgM, IgE, β1C and fibrin in the vessel walls was more frequently observed in intramural and perivascular sites than in intimal sites. IgM and fibrin were usually detected in intramural sites only. In large caliber arteries of the skin the intima was the main site of deposition of immune reactants, whereas, in small-caliber vessels, including capillaries, immune reactants were more often found in intramural and perivascular sites.

Key words: Cutaneous blood vessel; Immunoreactant; SLE; Immunofluorescence study

The dermal-epidermal junction has constituted a favorite site for immunofluorescence studies on affected skin in patients with systemic lupus erythermatosus (SLE). To our knowledge, only a few immunofluorescence studies have been published concerning vessels in SLE skin lesions (1, 2).

The present study concerns the deposition of various immunoglobulins, β1C, and fibrin in blood vessels of SLE skin lesions. 16 patients with SLE were investigated with the direct immunofluorescence technique. The findings obtained are presented in comparison with those obtained by light microscopy.

MATERIALS AND METHODS

Biopsies were taken from skin lesions in 16 patients with SLE. The main clinical laboratory findings in these patients are summarized in Table I. According to the diagnostic criteria for SLE established by the American Rheumatism Association, a diagnosis of SLE was confirmed in 15 and probable in 1 of these 16 patients.

Biopsies samples obtained were each divided into two parts; one was fixed in 10% formalin for hematoxylin-eosin (H & E) staining and the other was frozen in dry ice-acetone for subsequent immunofluorescence studies. The frozen materials were stored at −20 °C until 4–5 μm sections were prepared using a cryostat. The sections were placed on non-fluorescent slides and allowed to air-dry for 15 min. After washing 3–4 times in 0.005 M phosphate-buffered saline (PBS) at pH 7.2, for 5 min each, sections were allowed to react with fluorescein isothiocyanate (FITC) labeled antiserum in a moist chamber at room temperature for 30 min. Specimens were then washed 3 times for 5 min each in PBS, before sealing in glycerin buffer for observation under a fluorescence microscope (Chiyoda, Japan). Fujichrome ASA 100 film (Fuji Film, Japan) was used for microphotography. Sections were stained with H & E as soon as fluorescence was measurable. The immunofluorescence findings were compared with those obtained by light microscopy.

Conjugates

FITC-labeled anti human IgG, IgA, IgM and β1C (Behringwerke, West Germany) and FITC-labeled anti-human IgE and fibrin (Hyland, USA) were each diluted 10–20 fold before use. These conjugates had the following characteristics. Rabbit anti-human IgG: protein concentration (PC), 6.1 mg/ml; fluorescein to protein (F/P) ratio, 3.0; rabbit anti-human IgA: PC, 10.8 mg/ml: F/P ratio, 1.7; rabbit anti-human IgM: PC 12.3 mg/ml; F/P ratio, 1.1; rabbit anti-human β1C: PC, 2.3 mg/ml; F/P ratio, 1.1; goat anti-human IgE: PC, 10.7; F/P ratio, 3.0; rabbit anti-human fibrin: PC, 6.7 mg/ml; F/P ratio 4.7.

The blocking test was used to ascertain the specificity of these FITC-labeled antisera. Unlabeled antisera used in this test were the products of the same manufacturer as the labeled antisera and were diluted 2–5 fold before use. In the test it was as certained that fluorescence was extinguished with antisera of the same class and that no extinction or attenuation of fluorescence occurred with combinations of antisera of different classes.

RESULTS

For the purposes of the present study, blood vessels in the dermis and subcutaneous tissue were clas-

Table 1. *The main clinical findings of 16 cases of SLE*

F=female; M=male; ESR=erythrocyte sedimentation reaction; LE=lupus erythematosus; ANF=anti-nuclear factor; γ-gl=γ-globulin

Case No.	1	2	3	4	5	6	7	8
Age (y.)	36	14	25	31	14	28	33	26
Sex	F	F	F	F	F	F	F	F
Fever (°C)	38.4	38	40	39	36	38	38	38
ESR (1 h)	47	20	85	43	2	52	75	58
LE cell	+	+	+	−	−	−	+	+
ANF	1:512	1:16	1:512	1:64	−	1:32	1:256	1:64
γ-gl (g/dl)	1.9	2.0	2.7	1.7	1.3	0.7	1.7	2.4
Proteinuria	±	±	+	+	−	++	±	+
	9	10	11	12	13	14	15	16
Age (y.)	19	15	37	15	31	25	36	48
Sex	F	F	F	M	F	F	F	F
Fever (°C)	38	37	38	39	38	38	38	40
ESR (1 h)	40	70	26	121	14	51	15	85
LE cell	+	+	−	+	−	+	−	−
ANF	1:128	1:32	−	1:16	−	1:256	−	−
γ-gl (g/dl)	2.8	2.1	2.4	2.5	1.3	2.4	1.3	2.5
Proteinuria	++	+	−	±	±	+	−	−

sified into 2 types, small and large. The small type was defined as comprising arteriolae and venules including capillaries, while the latter type comprised arteries and veins, which were thicker in caliber than the former.

In these blood vessels, immune reactants were ordinarily deposited in a fine or coarse granular pattern. They were occasionally found forming a homogeneous pattern, lying beneath the endothelium at sites of probable thrombus or endothelial damage. The pattern of fluorescence in vessel walls was classified topographically into intimal, intramural, and perivascular. The results obtained are summarized in Tables II and III.

Table II depicts the relationship between the location and class of immune reactants in blood

Table II. *Relationship between vascular localization and immunoreactants*

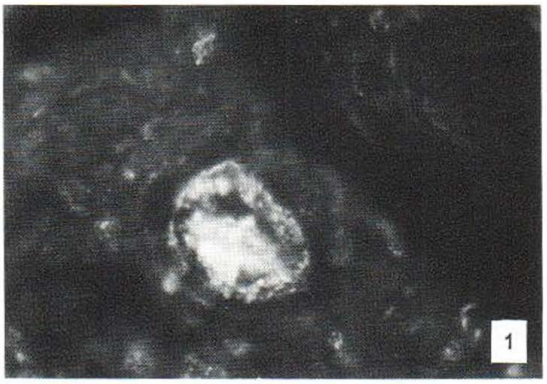
Vascular localization	Immunoreactants					
	IgG	IgA	IgM	IgE	βIC	Fibrin
Intimal	3[a]	3	4	2	1	5
Intramural	10	10	15	6	8	13
Perivascular	5	4	9	2	8	10

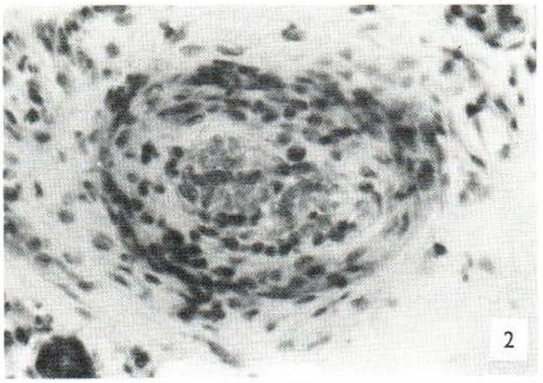
[a] Number of positive cases.

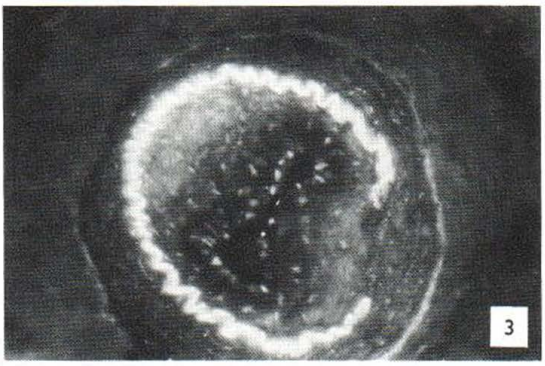
Table III. *Relationship between caliber of cutaneous vessels and vascular localization of FITC-labeled antisera*

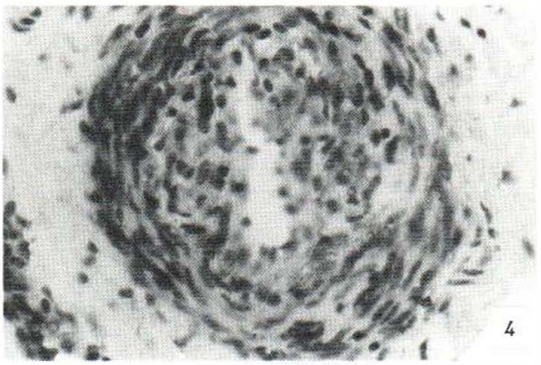
	No. of cases examined	Vascular localization of FITC-labeled antisera		
		Intimal	Intramural	Perivascular
Larger artery	7	3[a]	1	0
Larger vein	7	4	4	1
Smaller vessels including capillaries	16	7	16	13

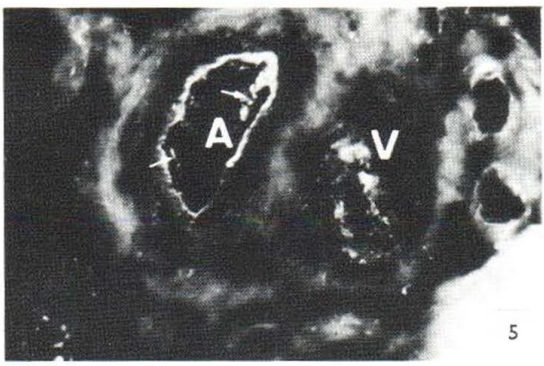
[a] Number of positive cases.











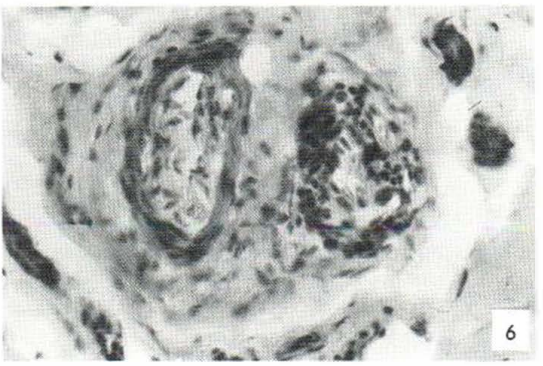


Fig. 1. IF positive finding of fibrin in wall of arteriole of lower dermis (case 14, foot, ×100).
Fig. 2. Higher magnification of arteriole shown in Fig. 1 (H & E stains). Cellular proliferation of intima and media (the same section as Fig. 1, ×200).
Fig. 3. IF positive finding of IgG seen in the nuclei of endothelial cells and portion of the media (case 14, foot, ×200).
Fig. 4. Finding after H & E staining of arteriole shown in Fig. 3. Marked cellular proliferation of intima and media (the same section as Fig. 3, ×200).
Fig. 5. IF positive finding of β1C in arteriole (A) (arrows) and venule (V) of lower dermis (case 9, hand, ×200).
Fig. 6. Finding after H & E staining of arteriole (proliferation of intima) and venule (panphlebitis) shown in Fig. 5 (the same section as Fig. 5, ×200).

vessels. The immune reactants were more often detected in intramural and perivascular areas than in intimal sites. IgM and fibrin, in particular, were often found in intramural sites.

Table III shows the relationship between the sites of deposition of immune reactants in blood vessels and the size of the vessels. In small vessels, including capillaries, fluorescence was detected more frequently at intramural or perivascular sites; whereas in larger vessels fluorescence was present mainly in intimal locations (positive in 3 of 7 cases). In one of these the deposition of immune reactants extend-

ed into the intramural region (Fig. 1). In these 3 cases the light-microscope findings (H & E stain) revealed pronounced intimal cellular hypertrophy, and mild proliferation of myogenic cells in the media (Fig. 2). In 2 of these cases there was thrombus formation and in 1, deposition of IgG in the nuclei of proliferating cells in the media (Fig. 3) and this H & E stains showed the marked cellular proliferation of intima and media (Fig. 4).

In large veins, fluorescence was detected at intimal or intramural sites in 4 of 7 cases. In one of these cases fluorescence was also seen in the perivascular region (Fig. 5). Light-microscopy (H & E stains) showed a marked cellular proliferation in the intima and media, with disruption of vascular architecture, i.e., a picture of panphlebitis (Fig. 6).

In small veins, fluorescence was located at intimal sites in 7, intramural sites in 16, and perivascular sites in 13 of 16 patients. This shows that fluorescence is more frequently detected in the intramural or perivascular sites of small vessels than in large vessels. It was difficult to distinguish between arteries and veins by observing H & E stained specimens, because of the marked enlargement and proliferation of cells in vessel walls in these cases. Pronounced infiltration by neutrophils and mononuclear cells into the perivascular region was also observed, as were occasional fibrin thrombi.

DISCUSSION

Fluorescence in the superficial blood vessels of cutaneous lesions associated with SLE displays a homogenous pattern at intimal sites and a granular pattern at intramural and perivascular sites (1).

In our present study, 16 cases of SLE showed that the patterns of fluorescence in blood vessels of the dermis and of subcutaneous tissue were generally fine granular or coarse granular. Such patterns were detected in intimal, intramural and perivascular locations. In probably damaged areas, a homogeneous pattern was observed at endothelial and subendothelial sites. Of the three different immune reactant deposition sites, intimal deposition was less common than intramural or perivascular deposition. This is obvious from Table II of the preceding section. The intima was the main location of immune reactants (3 of 7 cases) in large arteries (Fig. 1). These large arteries were found in the lower layer of the dermis and in subcutaneous tissue. Light microscopically, they exhibited the marked proliferation of endothelial cells (Fig. 2). In large veins, fluorescence was more frequently detected at intramural sites (4 of 7 cases) than in large arteries. In one of these cases fluorescence was observed in the perivascular region (Fig. 5). Light microscopy of these veins revealed a picture of panphlebitis with marked cellular proliferation of intima and media (Fig. 6).

In small vessels, including capillaries, fluorescence was more often located in intramural or perivascular sites than in large vessels. Light microscopically, these small vessels were affected by vasculitis, with a marked cellular infiltration of the perivascular region and, in some cases, formation of fibrinous thrombi.

Scott & Rowell (4) pointed to a relationship existing between the accumulation of immunoglobulins in cutaneous blood vessel wall and cellular infiltration of the perivascular area. They stated that deposition of immunoglobulins was frequent in the vascular lesions of various skin diseases, with marked changes in tissue (14 in 17 cases), though less frequent when tissue changes were of mild intensity (3 in 10 cases). Our study showed the presence of β1C and fibrin in blood vessels of SLE skin lesions. The deposits observed here may be considered to be immune complexes. There is experimental evidence that a major role is played by the leukocyte as well as by antigen antibody complexes and complement in the development of vasculitis (5). The leukocyte is induced to phagocytose immune complexes in the intima of vessels. It releases an enzyme which causes tissue damage and is believed to damage the basement membrane and internal elastic lamina of vessel walls (3). Immune complexes, accumulated in the intima, may be gradually transferred from the site of vascular damage to intramural and perivascular sites.

Our study showed that deposits of immune reactants are often found at intimal sites in large vessels and in intramural or perivascular locations in small vessels. This suggests a close relationship between the size of vessels and the site of immune complex deposition. The associated changes observed under the light microscope include thickening of the intima in large vessels and marked perivascular cell infiltration plus vasculitis with fibrosis in the small vessels. These changes appear to result from inflammatory reactions caused by the deposition of immune reactants in blood vessels.

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