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DIRECT IMMUNOFLUORESCENT FINDINGS IN SCLERODERMA SYNDROMES

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Abstract. Cutaneous direct immunofluorescent findings were examined in 78 patients who had either vascular scleroderma (group 1, 52 patients) or scleroderma with features of myositis or lupus erythematosus (group 2, 26 patients). Group 2 had higher antinuclear antibody levels, erythrocyte sedimentation rates, serum IgG concentrations, frequency of positive LE clot test, and rheumatoid factor activity. Ninety-two percent of group 1 (48 patients) had negative direct immunofluorescent findings, whereas 77 % of group 2 (20 patients) had positive findings at the basement membrane or in the blood vessels (or both). The 6 patients in group 2 who had negative immunofluorescent findings were all on systemic steroid therapy. Of the 17 patients in group 2 who had tests for antibody to extractable nuclear antigen, only 3 had high-titer antibody to ribonucleoprotein—a pattern characteristic of mixed connective tissue disease. Direct cutaneous immunofluorescence is proposed as a means of identifying those patients with scleroderma who may be steroid-responsive.

Key words: Vascular scleroderma; Myositis scleroderma; Lupus; Immunofluorescence; LE

Scleroderma is a disease of often subtle clinical findings characterized by fibromucinous change in the vascular endothelium, in skin, musculoskeletal system, and internal organs, notably the lung, esophagus, intestinal tract, heart, and kidney. Raynaud's phenomenon, tightness and hardening of the skin, sclerodactylia, telangiectasia, calcinosis, esophageal dysfunction, and interstitial pulmonary fibrosis are usual features of systemic vascular scleroderma.

There is a second group of patients with sclerodermatous findings who additionally present signs of inflammation clinically and serologically. Myositis, arthritis, lupoid features, and frequently edematous sclerosis are among the clinical characteristics of this group. This syndrome with overlapping symptoms suggestive of polymyositis, lupus erythematosus (LE), and scleroderma has been called "mesenchymal or inflammatory scleroderma" (15). Sharp et al. (13) have described such an overlap syndrome, called "mixed connective tissue disease", which is characterized serologically by high titer antibody to a ribonuclease-sensitive ribonucleoprotein (Rnp) component of an extractable nuclear antigen (ENA). Patterns of presentation have varied: patients may present initially with overlapping symptoms or may develop them sequentially (4, 12). Serologically, patients with such scleroderma tend to have hypergammaglobulinemia; high titers of antinuclear antibodies, which are frequently of a speckled pattern; rheumatoid factor; elevated muscle enzyme levels; and positive LE clot test. They may have a high-titer antibody to the Rnp component of ENA.

However, some patients with vascular scleroderma have anti-Rnp antibody, and conversely, some with overlap syndromes of scleroderma do not (12). Hence, antibody to ENA with specificity to the Rnp fraction cannot be regarded as a single test capable of diagnosing or confirming overlap syndromes. Although no controlled studies have been performed, the importance of identifying these syndromes appears to be the steroid sensitivity of the patients, especially early in their disease (9).

Previously, our group reported a series of 19 patients who had either progressive systemic vascular sclerosis or overlap scleroderma in whom cutaneous direct immunofluorescent studies had been performed (16). Patients with vascular scleroderma had negative immunofluorescent findings in involved, sun-exposed skin, whereas patients with immunoreactive scleroderma had either vascular or dermo-epidermal junctional immunoglobulin deposits, or both. The purpose of this paper is to report further on the clinical and cutaneous immunofluorescent findings in 78 patients.

Table I. *Patients with scleroderma syndromes*

	Vascular scleroderma negative immunofluorescence (48 patients)	Overlap scleroderma positive immunofluorescence (20 patients)
Mean age (yr.)	50	43
Male/female ratio	4/44	2/4
Mean duration of disease (yr.)	5	3.7
Mean sedimentation rate (mm in 1 h)	30	55
ANA (% positive)	53	100
Rheumatoid factor (% positive)	13.5	11.7
LE clot (% positive)	2.4	16.7

MATERIALS AND METHODS

Data were retrospectively collated on all patients with vascular or overlap scleroderma who had had direct cutaneous immunofluorescent examination of involved skin during the last 5 years. Punch biopsy specimens from involved skin of the dorsal hand or extensor wrist were immediately frozen in liquid nitrogen and stored at −70°C. The specimens were processed for direct immunofluorescent examination, whithin 1 week, by methods previously reported (1, 16). All patients had antinuclear antibody titers and erythrocyte sedimentation rates measured; and serum immunoglobulin levels, rheumatoid factor activity, and LE clot test were evaluated in most patients. Presence of hemagglutinating antibody to ENA was determined in 17 patients of group 1 and in 17 patients of group 2 (courtesy of Dr Frederic McDuffie's or Dr Gordon Sharp's laboratory); the specificity of antibody to the ribonuclease-sensitive Rnp fraction or the ribonuclease-resistant Sm fraction of ENA also was determined. The extent of disease with respect to pulmonary, gastrointestinal, or renal involvement was evaluated in almost all patients. Specific evaluation for myositis by serum enzyme levels, electromyography, and muscle biopsy was dictated by symptoms and signs of muscle dysfunction on physical examination.

RESULTS

Group 1: Vascular scleroderma

Characteristics of the 48 patients in group 1 who had negative cutaneous direct immunofluorescent findings were compared with those of the 20 patients in group 2 who had positive findings (Table I); the remaining 10 patients (4 from group 1 and 6 from group 2) will be discussed later. Two of the 48 patients had been on systemic steroid therapy (prednisone 20 mg orally, daily; prednisone 10 mg orally, four times per day) for an undetermined period of time when first evaluated at the Mayo Clinic; however, neither of these patients had previous symptoms suggestive of inflammatory scleroderma. All except 2 patients had Raynaud's phenomenon. Thirty-three patients had documented esophageal involvement; 24 had pulmonary involvement by chest roentgenogram and pulmonary function tests; and 7 patients had evidence of sclerodermatous small bowel or colon disease. One patient had Sjögren's syndrome, and one had vitiligo; 2 patients had alopecia areata, and one patient had a history of Hashimoto's thyroiditis with development of scleroderma 3 months after subtotal thyroidectomy. Four patients died: one each from suicide, renal involvement, and sclerodermatous gastrointestinal disease; the cause of the fourth death was unknown.

Antinuclear antibody titers were positive in 25 of the 47 patients tested (53%). Seven patients had speckled patterns, and 8 had nucleolar patterns; the other 10 had peripheral, mixed, or homogeneous patterns. Only 1 of the 41 patients tested had a positive LE clot test, which was negative on two subsequent occasions. Serum immunoglobulin levels were elevated in a minority of patients: 6 of the 48 patients (12.5%) had increased IgG concentrations; 17 patients (35.4%) had increased IgM levels; and 4 patients (8.3%) had increased serum IgA concentrations. Rheumatoid factor activity was present in 5 (13%) of the 39 patients tested; mean titer was 1:84. Levels of antinative DNA antibody and total hemolytic complement were normal in the 18 and 19 patients, respectively, tested. Antibody to ENA was detected in 3 of the 14 patients with immunofluorescent-negative vascular scleroderma in whom it was tested. One of these patients had antibody to the Rnp component of ENA. This patient was a 22-year-old woman with an 8-year history of Raynaud's phenomenon and progressive sclerodermatous acral changes and a 1-year history of dysphagia, with esophageal motility studies confirming sclerodermatous involvement. She had no symptoms or findings suggestive of myositis or lupus. Laboratory data including erythrocyte sedimentation rate, LE clot, rheumatoid factor, total hemolytic complement, and antibody to native DNA were all normal or negative. She had a high-titer antinuclear antibody (1:4 096) in a speckled pattern in addition to polyclonal hyperglobulinemia. The other 2 patients had antibody to ENA fractions that were not characterized as ribonuclease-sensitive or ribonuclease-resistant, but were considered as unknown.

Five patients had nonspecific cutaneous immunofluorescent findings: 4 had cytoids at the basement membrane (IgM, IgG, C3; IgM, C3; IgM alone), and one had fibrin in the blood vessels. None of these patients had signs or symptoms suggestive of myositis or lupus. Antinuclear antibody was negative in 4 of the 5 tested, and antibody to ENA was negative in the 3 patients tested.

Among the 52 patients with vascular scleroderma comprising group 1 were 4 with cutaneous immunoglobulin deposition (Table II). All 4 were females; their mean age was 39 years, and mean duration of symptoms, 9 years. None had received steroids systemically. All had Raynaud's phenomenon; 2 had documented esophageal involvement, and one had pulmonary involvement. None had gastrointestinal or renal involvement, and none had symptoms of myositis or lupus.

The first patient had Hashimoto's thyroiditis diagnosed at the time of evaluation; antithyroglobulin antibody titer

Table II. *Positive direct immunofluorescence in patients with vascular scleroderma*

Case	Sex and age (yr.)	Duration of symptoms (yr.)	ESR (mm in 1 h)	Antinuclear antibody (titer)	Rheumatoid factor (titer)	LE clot test	Immunoglobulin concentration (mg/ml)			Immunofluorescence		
							IgG	IgM	IgA	Basement membrane zone	Blood vessels	Cytoids
1	F, 61	15	21	1 : 256 mixed	Neg.	Neg.	11.13	1.86	3.82	IgM	Neg.	IgM
2	F, 23	8	10	Neg.	Neg.	Neg.	6.27	4.04	0.30	IgM	Fibrin	IgM
3	F, 28	6	7	Neg.	Neg.	Neg.	7.37	1.27	0.84	IgM	Neg.	IgM
4	F, 43	8	13	1 : 1024 nucleolar	Neg.	Not done	Not done	Not done	Not done	IgM	Neg.	Neg.

was markedly elevated (1 : 25 600). Additionally, she had Sjögren's syndrome. The remainder of her evaluation was indicative of sclerodermatous pulmonary and esophageal involvement; antinative DNA antibody and total hemolytic complement levels were normal. Antibody to ENA was not determined. The second patient had a family history of systemic LE (maternal grandmother). Evaluation was unremarkable except for acrosclerosis and subcutaneous calcification. Antinative DNA antibody levels and total hemolytic complement were normal. Antibody to ENA was negative. The third patient had vitiligo of the hands and feet in addition to findings of acrosclerosis and esophageal scleroderma. Antinative DNA antibody levels and total hemolytic complement were normal. Antibody to ENA was negative. The fourth patient had a history of atopic eczema. Findings were limited to acrosclerosis with digital ulceration and subcutaneous calcification; results of esophageal, pulmonary, and gastrointestinal evaluations were normal. Antibody to ENA was negative.

All 4 patients had IgM deposition at the basement membrane; 3 had granular patterns. Weak staining was present in one (case 4). Other findings were cytoids in 3 of the 4, and blood vessel fibrin deposition in one (case 2).

Group 2: Overlap or inflammatory scleroderma

Group 2 consisted of 26 patients who presented with sclerodermatous cutaneous involvement and clinical or laboratory (or both) features of LE, dermatomyositis, or polymyositis. Various patterns of disease were noted in this group: onset of scleroderma with myositis, scleroderma followed by myositis months to years later, and scleroderma with or without myositis preceded or followed by development of lupoid features such as lupus panniculitis or discoid or systemic LE.

Twenty of the patients who presented with inflammatory scleroderma had positive direct cutaneous immunofluorescent findings in involved sclerodermatous skin (Table III). Nine of the 20 had a diagnosis of sclerodermatomyositis at the time of biopsy; others had a diagnosis of inflammatory scleroderma. Prednisone was being used in 4 patients; mean duration of treatment was 1.2 years (range 1 month to 2½ years) with a mean daily oral dose of 25 mg. No patient was receiving cytotoxic therapy. There were 4 males among the 20 patients; average age was 43 years. Fifteen of the 20 patients had Raynaud's phenomenon. All 20 had sclerodermatous esophageal disease; 17 had pulmonary involvement, with severe pulmonary hypertension in 1 (case 9). Gastrointestinal involvement was present in 4 of the 9 patients checked. Sixteen patients had myositis involving the proximal musculature; it was documented by neurologic examination, muscle enzyme study, and electromyography or muscle biopsy (or both) in 13 patients. Features of lupus were present in 6 patients and included lupus panniculitis (case 7), photosensitive cutaneous eruption (cases 2 and 6), discoid LE 10 years (case 16) and 16 years (case 14) before onset of inflammatory scleroderma, systemic LE with lupus nephritis 10 years before onset of scleroderma (case 12), and lupus nephritis (case 19). One patient (case 16) died from cardiac and renal sclerodermatous involvement. Two patients within this group had vitiligo (cases 10 and 11); 3 patients had Sjögren's syndrome (cases 1, 4, and 8); and 3 patients had thyroid disease (Hashimoto's thyroiditis in cases 7 and 20 and multinodular goiter in case 10).

Antinuclear antibody was present in all 20 patients; the titers tended to be higher in this group of patients. LE clot test was positive in one (case 12); occasional LE cells were observed in 2 (cases 7 and 13), and rosettes and nucleolysis were seen in 2 other patients (cases 14 and 17). Low-titer rheumatoid factor was present in 2 patients (cases 19 and 20). Unlike patients in group 1, patients in group 2 frequently had elevated serum IgG and IgM levels. Serum IgG level was elevated in 11 of 17 patients; IgM was elevated in 10 of 17 patients. Antibody level to native DNA was normal in 8 of 9 patients in whom it was measured and was increased to 5.07 μg/ml in one (case 14) (normal range up to 0.82 μg/ml). Total hemolytic complement was normal in 8 patients and decreased in 2. Antibody to ENA was measured in 12 patients and was present in 6. The antibody could be further characterized to the Sm fraction in one patient (case 5), who had inflammatory myopathy and esophageal and severe pulmonary sclerodermatous involvement, and to the Rnp fraction in 2 (cases 19 and 20). In 3 patients (cases 4, 6, and 7), antibody to unidentified nuclear antigens was present and antibodies to Rnp and Sm fractions were negative.

Cutaneous immunofluorescent deposits were found at the basement membrane in 16 of the 20 patients, as described in Table III; the pattern of fluorescence was granular. Blood vessel immunofluorescence was present in 6 patients, 4 of whom had no dermo-epidermal immuno-

Table III. *Positive direct immunofluorescence in patients with overlap scleroderma*

Case	Sex and age (yr.)	Duration of symptoms (yr.)	ESR (mm in 1 h)	Antinuclear antibody (titer)	Immunoglobulins (mg/ml)			Immunofluorescent findings			
					IgG	IgM	IgA	Basement membrane zone	Blood vessels	Cytoids	Other
1	F, 48	1	25	1 : 1 024 nucleolar	9.8	2.9	3.92	IgM	...	...	...
2	F, 34	3	42	1 : 1 024 speckled	14.45	1.54	2.09	IgM, C3	IgM, C3, fibrin	...	...
3	F, 36	1	Not done	1 : 640 speckled	Not done	Not done	Not done	IgM, fibrin	...	...	...
4	M, 52	5	55	1 : 1 024 nucleolar	19.18	2.07	3.67	...	IgM	IgM	...
5	F, 64	2	68	1 : 4 096 speckled	29.0	1.26	3.0	IgM	...	...	IgG epidermal, nuclear
6	M, 58	12	107	1 : 512 mixed	31.6	0.34	3.97	C3, fibrin	...	IgM	...
7	F, 45	15	85	1 : 256 mixed	24.54	2.19	2.38	IgM	...	...	...
8	F, 66	2	62	1 : 16 384 nucleolar	10.11	3.24	1.35	IgG	...	...	IgG epidermal, nuclear
9	F, 23	4	40	1 : 64 nucleolar	16.18	1.94	1.19	...	IgM, fibrin	...	...
10	F, 70	1/4	65	1 : 65 536 nucleolar	Not done	Not done	Not done	IgM	...	IgM	...
11	F, 38	3	40	1 : 4096 speckled	14.77	3.0	1.08	IgM, C3	...	...	...
12	F, 32	4	73	1 : 32 mixed	Not done	Not done	Not done	IgG, IgM	...	...	IgG epidermal, nuclear
13	F, 41	2	85	1 : 1 024	39.7	2.39	5.3	IgM	IgM	...	...
14	F, 33	1	67	1 : 256 speckled	17.5	0.35	1.65	IgM, C3	...	...	...
15	F, 27	5	21	1 : 32 speckled	13.33	1.79	3.66	IgM	...	...	...
16	F, 51	3	52	1 : 640 mixed	8.4	0.73	2.2	IgM	...	...	...
17	F, 59	1	30	1 : 1 024 mixed	11.2	3.42	2.28	IgM	...	...	...
18	M, 38	1	41	1 : 32 speckled	11.7	0.88	1.5	IgM, fibrin	...	...	...
19	M, 13	1	42	1 : 256 speckled	15.49	0.62	2.7	...	C3, fibrin	...	...
20	F, 33	7	49	1 : 4096 speckled	51.7	3.22	0.91	...	C3, fibrin	...	...

fluorescence. IgM cytoids at the basement membrane zone were also found in 3 patients, 2 of whom had basement membrane immunofluorescent findings. IgG epidermal nuclear staining was present in 3 patients, of whom one had antibody to the Sm fraction of ENA and a second who had no antibody to ENA. The third patient did not have an anti-ENA antibody test performed. Those with epidermal antinuclear immunofluorescent staining also had basement membrane zone staining with IgM in one case, IgM and IgG in another, and IgG alone in the third.

Data were collected on 6 patients in group 2 who did not have positive cutaneous immunofluorescence (Table IV). The outstanding feature of this group was systemic steroid treatment in all patients; average duration of therapy was 5.9 years, and none had been on steroid therapy for less then 4 months. Two of the 6 were males. Mean age was 57 years; mean duration of symptoms was 3.4 years and was comparable to that in the remainder of group 2. Three of the patients (cases 2, 3, and 6) had esophageal involvement, and 3 (cases 2, 3, and 4) had pulmonary involvement. Gastrointestinal involvement was present in one (case 3). All except one (case 1) had Raynaud's phenomenon. Inflammatory myositis involving proximal musculature and confirmed by muscle biopsy or electromyography

Table IV. *Negative direct immunofluorescence in patients with overlap scleroderma*

Case	Sex and age (yr.)	Duration of symptoms (yr.)	ESR (mm in 1 h)	Antinuclear antibody (titer)	Rheumatoid factor (titer)	LE clot test	Immunoglobulins (mg/ml)			Extractable nuclear antigen
							IgG	IgM	IgA	
1	F, 47	3/4	6	1:64 speckled	Neg.	Neg.	Not done	Not done	Not done	Neg.
2	M, 63	7	38	Neg.	Neg.	Neg.	9.72	1.58	2.6	Neg.
3	F, 53	5	55	1:64 speckled	Not done	Neg.	Not done	Not done	Not done	Not done
4	M, 59	3	49	1:128 speckled	1:10240	Not done	18.5	3.55	5.0	Neg.
5	F, 72	1/4	61	1:2048 speckled	Neg.	Neg.	7.6	1.53	1.49	Pos.
6	F, 50	4	52	1:16384 speckled	Neg.	Neg.	15.56	2.54	2.79	Pos.

(or both) was present in all. In 3 patients (cases 1, 2, and 4), cutaneous sclerosis and myositis were present from the onset; cutaneous and esophageal sclerodermatous involvement preceded myositis symptoms in 2 patients (cases 5 and 6) by 1 year and 3 months, respectively.

Lupoid features were present in one (case 3). She had migratory arthritis, fever, markedly elevated erythrocyte sedimentation rate, pleuritic pain, pericarditis, and an acute, transient paranoid schizophrenic episode successfully treated with steroids 16 years before sclerodermatous disease was diagnosed. Intermittently she had a sun-sensitive butterfly rash and arthralgias responsive to steroid therapy since then, including the time after onset of sclerodermatous cutaneous, esophageal pulmonary, and gastrointestinal disease. One patient (case 6) had Hashimoto's thyroiditis diagnosed at the time of initial evaluation. There was one death (case 3) in this group—that from myocardial infarction.

Antinuclear antibody was present in 5 of the 6 patients, with a speckled pattern in all. Four patients had serum immunoglobulin levels determined: IgG was elevated in 2; IgM was elevated in all 4; and IgA was elevated in one. Significant rheumatoid factor activity was present in one (case 4); he did not have clinical signs of rheumatoid arthritis. LE cells were not present in the 5 tested; antinative DNA antibody and total hemolytic complement levels were normal in the 4 patients tested. Cutaneous immunofluorescence in all was negative, with nonspecific clumped IgM cytoids found in one patient (case 6) at the dermoepidermal junction. Antibody to ENA was present in 2 of the 5 patients tested, with Rnp characteristics in one (case 6) and the other being positive for an unidentified nuclear antigen and negative for Rnp and Sm.

DISCUSSION

Clinically, the patients with overlap scleroderma in this study tended to have their disease for a shorter time than did those patients with vascular scleroderma, as has been noted previously (16). Age was not significantly different in the two groups. Erythrocyte sedimentation rates and serum IgG concentrations were higher in those with more inflammatory disease; LE clot tests were more frequently positive and antinuclear antibody was more frequently present in this group. However, no one laboratory parameter can differentiate with certainty those patients with mixed vs. overlap scleroderma. The presence of antibody to ENA does not strictly correlate with presence of an overlap syndrome; this has been pointed out in the studies of Sharp et al. (12), Parker (9), Farber & Bole (2), and Hamburger et al. (6).

Cutaneous direct immunofluorescence can be helpful in detecting patients with overlapping features and in differentiating them from patients with vascular scleroderma, many of whom may have nonspecific symptoms suggestive of overlap but not borne out by laboratory testing. The absence of immunofluorescent findings in involved exposed skin in 92% of the patients with vascular scleroderma in this study is striking. The 4 patients with vascular scleroderma and positive findings tended to have symptoms for a longer time than did other patients with noninflammatory scleroderma, and they also tended to be younger. Serologically, they did not have higher serum immunoglobulins or higher antinuclear antibody levels. Three of the 4 patients had diseases often noted as autoimmune or had family histories of such disease. Whether this is pertinent with respect to their cutaneous immunofluorescent findings or whether symptoms of an

Table V. *Antibody to extractable nuclear antigens in scleroderma syndromes*

Type	Direct immuno-fluorescence	No. tested	Antibody to extractable nuclear antigen	Ribo-nucleo-protein (Rnp)	Ribonuclease-resistant fraction (Sm)	Other
Vascular scleroderma	Pos.	3	0	0	0	0
	Neg.	14	3	1	0	2
Overlap or inflammatory scleroderma	Pos.	12	6	2	1	3
	Neg.	5	2	1	0	1

overlap syndrome will develop later is not known; further follow-up of these patients is required. Biopsy of sun-exposed, chronically inflamed skin is another possible explanation for the positive immunofluorescent findings in these patients; however, all patients in each group had sun-exposed skin examined.

Seventy-seven percent of patients who had scleroderma and overlapping features suggestive of polymyositis, dermatomyositis, or LE had cutaneous immunofluorescent findings present at the basement membrane zone or in blood vessels. The patterns of immunofluorescent staining were not diagnostic of or pathognomonic for a type of scleroderma; similar patterns can be seen in systemic or discoid LE and various vasculitides. However, these patterns have not been noted in systemic scleroderma without inflammatory symptoms. That the immunofluorescent staining is specific is suggested by the lack of such findings in similar skin biopsy specimens from patients with vascular scleroderma who had disease for a longer duration and who would be expected to have more sclerosis, telangiectasia, and vascular change.

That 23% of patients with overlap scleroderma had negative cutaneous immunofluorescent findings is disconcerting; however, all of these patients had been on systemic steroid therapy. The response of cutaneous immunofluorescent findings to steroid therapy has not been thoroughly evaluated. Gilliam et al. (3) have reported loss of fluorescence at the basement membrane zone in the skin of patients with lupus after therapy with cyclophosphamide. Further studies regarding effects of duration of lesion, systemic therapy and sites of skin biopsied are indicated.

Thirty-four patients had tests for antibody to ENA, an acidic nuclear protein extracted by homogenization with neutral saline (Table V). In 23 patients, no antibody was present, and in 6, antibodies to unidentified fractions were present. In one patient with inflammatory scleroderma who had esophageal and severe pulmonary involvement and myopathy, an antibody to the Sm fraction of ENA was present; she had no evidence of LE. Three patients with overlap scleroderma and one patient with vascular scleroderma had antibody to the Rnp fraction of ENA. The significance of these antibodies to nuclear fractions has not been well established. Those with specificity to Sm are more likely to occur in patients with typical systemic LE, although they have been found in patients with noninflammatory scleroderma, with dermatomyositis, and with overlapping symptoms of progressive systemic scleroderma and systemic LE (2, 12). Antibody with specificity to Rnp has been found in mixed connective tissue disease but also in scleroderma with or without evidence of inflammation (2, 6, 8, 9, 12, 14). That the antibody to Rnp antigen is the same in scleroderma as in mixed connective tissue disease has been demonstrated by immunodiffusion studies.

That 9 of our patients with overlap scleroderma did not have antibody to ENA is in keeping with reports from a number of authors, who have noted its absence in overlap syndromes (2, 12). It is not clear whether patients with clinical and serologically mixed connective tissue disease differ from those patients who are clinically similar but who are serologically negative for ENA. Those with anti-Rnp antibody tend to have lower antinative DNA antibody levels, less frequent decreased total hemolytic complement levels, less frequently impaired renal function, and an increased incidence of Raynaud's phenomenon and cutaneous lesions (6). Steroid responsiveness has been attributed to those patients with positive ENA, but this too is variable in our experience (15).

Hamburger et al. (6) examined the cutaneous immunofluorescent findings of 46 patients with systemic LE. Of the 25 patients with anti-Rnp or anti-Sm antibody, 22 had immune deposits at the basement membrane zone; of the 21 without anti-ENA antibody, 11 had immune deposits at the dermo-epidermal junction. Similar to our efforts in the present study, they were unable to find a correlation between cutaneous immunofluorescence and presence of anti-ENA antibody. No descriptions of the patients examined were given.

Recently, Prystowsky et al. (10) noted two patterns of cutaneous immunofluorescence in patients with scleroderma of scleroderma overlap syndromes. In 6 patients with scleroderma and 2 patients with scleroderma–systemic LE, epidermal nucleolar IgG deposition in biopsy specimens of clinically normal sun-exposed skin was observed and interpreted as secondary to high-titer nucleolar antinuclear antibody. This finding was rare in the experience of the authors; these 8 patients were the only ones with this pattern among 3453 whose skin specimens were examined for immunofluorescence during a 5-year period. The 2 patients with scleroderma–systemic LE also had either IgM or IgG at the basement membrane zone.

Epidermal speckled nuclear IgG deposition was the second pattern observed (11). Among 46 patients with this finding in clinically normal skin, 81% had antibody to ENA. Those with antibody of Rnp specificity (86%) had a high incidence of Raynaud's phenomenon with overlapping features of systemic LE, scleroderma, or dermatomyositis or some combination of these. Those with antibody of Sm specificity (14%) had systemic LE. No data regarding the frequency of epidermal speckled pattern in patients with overlap syndromes were presented. Thirty-five percent of the patients in that study also had a positive lupus band test; however, their clinical characteristics were not given. Winkelmann et al. (17) reported direct immunofluorescence of nuclei as well as band immunofluorescence in florid or mixed cases of dermatomyositis. Izuno (7) reported 4 of 5 patients with mixed connective tissue disease who had both positive band and speckled epidermal nuclear fluorescence. In a previous paper, Gilliam & Prystowsky (5) noted this same speckled epidermal nuclear pattern in 15 patients with mixed connective tissue disease, only 1 of whom had findings suggestive of scleroderma. We believe the low incidence of nuclear fluorescence in our study relates to different patterns of patient selection in the several studies. Our low ENA incidence confirms this impression too.

Our evaluation of direct immunofluorescence in patients presenting with clinical scleroderma indicates clearly a positive and a negative group with relation to myositis and inflammatory features correlating with the presence of positive findings. Such data, added to results of serologic testing, staging of scleroderma, and presence of associated autoimmune disease, give a pattern of scleroderma disease in which the cautious use of corticosteroid therapy or antimalarial drugs may be of benefit.

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Section of Publications
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