Clinical Significance of Serum Properdin Levels and Properdin Deposition in the Dermal-Epidermal Junction in Systemic Lupus Erythematosus

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ABSTRACT 61 biopsies of normal skin from the deltoid area and lesional skin from various sites from 48 patients with systemic lupus erythematosus (SLE) were studied for the presence of properdin, C3, C4, and immunoglobulins (IgG, IgM, and IgA) in the dermal-epidermal junction (DEJ) using direct and indirect immunofluorescence. Properdin was present in 50% of normal and 40% of lesional skins. Properdin was present without C4 in only 2 of 38 nonlesional skin biopsies and in only 2 of 20 lesions. There was no significant difference in incidence of deposition of any of the six proteins studied between nonlesional and lesional skin.

The frequency of deposition of each of the proteins correlated with clinical disease activity. The presence of proteins in the DEJ did not correlate with the presence of active renal disease at the time of biopsy nor with previously documented active nephritis. In addition, no other single clinical manifestation correlated with the presence of DEJ deposition of any protein studied. IgA was not demonstrated in the DEJ of nonlesional skin of 16 patients in remission and was present in 7 of 23 patients with active disease ($P < 0.05$). Deposition of properdin in lesional skin correlated with the presence of extracutaneous disease activity ($P < 0.05$).

Analysis of serologic studies on serum obtained at the time of biopsy revealed a statistically significant correlation between C4 and C3 ($r = 0.67$). This correlation was stronger than that between properdin and C3 ($r = 0.49$) which in turn was stronger than that between properdin and C4 ($r = 0.37$). Titer of antinuclear antibody and percent of DNA binding correlated better with C4 levels than with properdin levels. Serum properdin levels were significantly lower in patients with active disease than in those in remission ($P < 0.05$). Serum properdin levels were significantly lower in patients with properdin deposits in lesional skin than in those without properdin deposits.

The data suggest that both alternative and classical pathways are activated in patients with clinically active SLE.

INTRODUCTION

The complement system is a group of serum proteins known to play a role in the inflammatory response (1). There is ample evidence to implicate the classical pathway of complement activation in the production of tissue damage in systemic lupus erythematosus (SLE)[1] (2). The initiating event in this sequence is presumed to be the formation of immune complexes involving DNA and anti-DNA antibodies (3, 4).

The existence of an additional pathway capable of activating the terminal complement components (C3, C5–C9) without consumption of the early classical components (C1, C4, C2) was first suggested by Pillemer et al. (5). Independent investigations in several laboratories have recently confirmed the existence of such a group of proteins which is now known as the alternative or properdin pathway (1, 6, 7). This sequence may be activated in vitro by a variety of substances including complex polysaccharides, aggregated IgA (8), and C3 nephritic factor (C3NeF) (9). Although the precise nature of the pathway is not yet completely clear, the

This paper was published in part in abstract form: 1975. *Clin. Res.* 23: 412A.

Received for publication 23 May 1975 and in revised form 2 September 1975.

[1] *Abbreviations used in this paper:* ANA, antinuclear antibody; C3NeF, C3 nephritic factor; DEJ, dermal-epidermal junction; FITC, fluorescein isothiocyanate; SLE, systemic lupus erythematosus.

following proteins have been identified: properdin, a highly basic gamma globulin with a mol wt of 186,000 (10); factor A, a hydrazine-sensitive protein identical to C3 (11); factor B, a glycine-rich β-glycoprotein identical to C3 proactivator which in its active form participates in the cleavage of C3 (12, 13); and factor D, a low molecular weight proteolytic enzyme capable of cleaving factor B to its active form (14). This system requires magnesium. A feedback loop has been described in which C3b produced by the classical pathway interacts with factors B and D to promote further cleavage of C3 (15, 16). Recent data suggest that properdin may also increase the efficiency of this feedback loop (17).

Patients with SLE and nephritis have deposits of immunoglobulins and complement components in the mesangium and glomerular basement membrane (3). Similar deposits have been described in the dermal-epidermal junction (DEJ) of normal and lesional skin of SLE patients (18–21). Several authors have correlated the presence in normal skin of immunoglobulins and complement (the "lupus band test") with lupus nephritis (21, 22). Others have found no such correlation (23, 24).

Westberg et al. demonstrated that properdin, in addition to proteins of the classical pathway, was present in the glomerular basement membrane of 3 of 13 SLE patients (25). We have confirmed this observation and have shown that properdin is also present in the DEJ of skin lesions from SLE patients (26). Provost and Tomasi demonstrated properdin in the DEJ of normal skin from several SLE patients (27) and recently Jordon et al. found both properdin and factor B in clinically normal and lesional skin (28). Additional evidence for activation of the alternative pathway in patients with SLE is provided by studies showing that serum levels of properdin and factor B may be reduced (29–31) and properdin and factor B metabolism increased in lupus nephritis (32, 33).

In this report, we describe the presence of properdin in both lesional and normal skin from SLE patients and demonstrate that reduced serum properdin levels and deposition of properdin in the DEJ of skin lesions occur during periods of clinically active disease. Correlations between serum antinuclear antibody titers, antiDNA antibodies, C3 and C4, immunofluorescent findings in the DEJ, and clinical disease activity suggest that both the alternative and classical pathways are involved during clinical disease activity.

METHODS

Patients and controls. 48 patients with SLE whose ages ranged from 10 to 65 yr (mean 34.2 yr) were studied. There were 4 males and 44 females, and the group consisted of 44 whites, 2 blacks, and 2 Latin Americans. All patients had multisystem disease and fulfilled the preliminary criteria for the classification of SLE (34). Patients were seen at the Rheumatic Diseases Clinics at the University of Connecticut Health Center (Hartford, Conn.) and at affiliated hospitals. In 29 patients clinically normal skin was biopsied; in 12, lesional skin; in 10, clinically normal and lesional skin were obtained simultaneously. Three of the patients were studied on two separate occasions. Clinical activity was assessed according to the guidelines of Rothfield and Pace (35) on the basis of history and physical findings alone as follows: 0—no clinical evidence of disease activity (remission); 1+—active disease in only one system; 2+—active disease in more than one system without fever or in one system with fever; 3+—active disease in two or more systems with fever. The presence of either hematologic abnormalities (white blood count < 4,000, platelet count < 100,000, or hemolytic anemia) or renal disease did not alter the assessment of clinical disease activity. Although renal disease was present in 14 patients (27.5%), it was active in only 5 at the time of skin biopsy. Active renal disease was defined as the presence of five or more red blood cells per high power field, cellular casts, and/or, increasing proteinuria. Renal biopsies performed before this study on these five patients had shown diffuse proliferative lupus nephritis in two patients and membranous lupus nephritis in one (36). Two had never been biopsied. Previous renal biopsies had been performed on eight of nine patients with inactive renal disease. Of these, four had focal proliferative lupus nephritis, three had diffuse proliferative, and one had membranous lupus nephritis.

At the time of skin biopsy, 42 patients were receiving corticosteroids in doses ranging from 5 to 80 mg of prednisone or its equivalent per day. The mean daily dose was 18.2 mg. 18 patients were receiving antimalarials at the time of biopsy in a dose of either 200 or 400 mg of hydroxychloroquine or its equivalent per day. Only two patients, both with diffuse proliferative lupus nephritis, were receiving immunosuppressive therapy in addition to corticosteroids. One was taking cyclophosphamide, 75 mg daily and had clinically inactive renal disease, mild azotemia, (creatinine 2.1 mg/100 ml) and "0" clinical disease activity. The other was receiving azathioprine, 50 mg daily, and had an active sediment, a serum creatinine of 1.4 mg/100 ml, and "1+" clinical disease activity (mucosal ulcers).

Normal skin was obtained from 5 healthy individuals and 13 patients with a variety of diseases. These included one healthy, serologically normal sister of an SLE patient, four patients with musculo-skeletal symptoms without definite evidence of rheumatic diseases, one patient with fever of unknown origin, one with chronic active hepatitis, two with rheumatoid arthritis, one of whom had a positive LE cell test, one with sun-sensitive eruption, one with a history of cutaneous vasculitis, and one with Raynaud's disease. None of these control patients had a positive antinuclear antibody test (ANA) or any serologic abnormalities except for the one patient with rheumatoid arthritis and a positive LE cell test.

Lesional skin was obtained from 24 patients with the following diagnoses: systemic sclerosis (7), psoriasis (1), cutaneous vasculitis (4), Weber-Christian Disease (1), urticaria (3); eczema (2), nonthrombocytopenic purpura (2), erythema multiforme and juvenile rheumatoid arthritis (1), poikiloderma (1), rheumatoid arthritis and lichen planus (1), and nonspecific dermatitis (1).

Skin biopsies. Punch biopsies, 4–6 mm in diameter, were taken from normal and lesional areas. Most normal skin biopsies were obtained from the deltoid region. Biopsies of

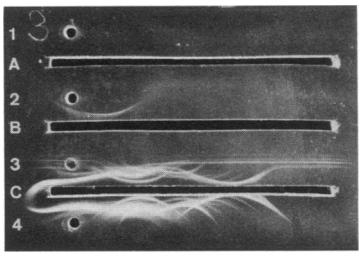

FIGURE 1 Immunoelectrophoresis of antiproperdin serum. 1 and 3 are normal human serum; 2 and 4 normal human plasma; A is rabbit antiproperdin; B is rabbit antifibrinogen; C is goat anti-whole human serum. The arc between B and 2 is not present between A and 2 indicating the absence of antifibrinogen in the antiproperdin serum.

lesions were taken from clinically active areas. All samples were bisected immediately. One portion was fixed in 10% formalin for routine processing; and the other segment was snap frozen in isopentane and dry ice and transferred to Cryoform (Ames Co., Elkhart, Ind.). Tissue was sectioned at 4 μm thickness within 72 h, and cut sections were stored at -70°C until use.

Serologic methods. All patients had a battery of hematological and serological studies, and a urinalysis at the time of skin biopsy. The indirect fluorescent antibody technique for antinuclear antibodies (ANA) was carried out using mouse liver as previously described (37). Patients' sera were tested undiluted and at serial twofold dilutions. Fluorescein isothiocyanate-(FITC) conjugated goat anti-human IgG (gamma chain specific) was purchased from Antibodies, Inc., Davis, Calif.

Levels of complement components C3 and C4 were determined by single radial immunodiffusion on commercially prepared plates (Behring Diagnostics, American Hoechst Corp., Somerville, N. J.). All determinations were done in duplicate on the same lot of plates by the same individual and results were averaged. Discordant results were repeated.

Antibodies to native, double-standard DNA were measured in each test serum by modification of the Farr technique (38). Tritiated DNA was prepared from a thymidine minus strain of *Escherichia coli* K12 as described previously. Column chromatography of this material on hydroxyapatite confirmed the absence of any contamination by denatured, single-stranded DNA (39). Binding of more than 50% of the labeled DNA by the test serum using this technique has been shown to be quite specific for active SLE (38).

Properdin levels were determined by electroimmunoassay (40) using a water-cooled electrophoresis apparatus (MRA Corp., Boston, Mass.) and rabbit antihuman properdin.[2] Properdin concentrations were expressed as a percentage of the normal pool described below. All sera were run on duplicate plates and results averaged. Maximum variation in properdin concentration on a single sample determined in this manner was ±5%. In 24 patients comparison of this technique with the solid-phase radioimmunoassay (41) has shown a high correlation ($r = 0.90$) between the two methods.[3]

Control sera used for all of the serological tests were obtained from 49 normal individuals ranging in age from 17 to 69 yr (mean 35.7). All of the individuals in this group were white and there were 4 males and 45 females.

Skin immunofluorescence. Immunofluorescent studies were done within 5 days of the biopsy, although tissue remained in satisfactory condition for up to 1 yr at -70°C. The following FITC-conjugated goat antisera were used: IgG (Antibodies, Inc., Davis, Calif.), Molar F/P ratio 3.3, dilution 1:4. IgM (Hyland Div., Travenol Laboratories, Inc., Costa Mesa, Calif.), F/P ratio 2.6, dilution 1:16. IgA (Hyland Lab., Costa Mesa, Calif.), F/P ratio 3.3, dilution 1:16. C3 (Meloy Laboratories, Inc., Springfield, Va.), F/P ratio 1:8, dilution 1:8. C4 (Meloy Lab., Springfield, Va.), F/P ratio 1.2, dilution 1:8. These dilutions produced the best resolution between specific apple-green fluorescence in the DEJ and background fluorescence of known positive control skin. All these conjugates produced a single arc on immunoelectrophores against normal human serum. The anti-C3 gave one line against aged human serum (3 days at 37°C) reacting only with the C3c antigen, and not with C3d. Appropriate positive and negative control slides were run with each conjugate.

Properdin was detected by means of the indirect immunofluorescent technique (22), using the same rabbit antiproperdin employed in the properdin assay and FITC conjugated goat antirabbit IgG (Microbiological Associates, Bethesda, Md.), F/P ratio 1.4. Both antisera were used at 1:16 dilution. Specific immunofluorescence could be abolished completely by incubating the antiproperdin with purified properdin (42) before incubation with the tissue. Furthermore, incubation of FITC goat antirabbit IgG alone with tissue produced no specific fluorescence. The antiproperdin did not react with fibrinogen on immunoelectrophoresis using plasma as antigen (Fig. 1).

All slides were viewed on a Leitz Ortholux II microscope (E. Leitz, Inc., Rockleigh, N. J.) equipped with a Ploem vertical illuminator. Illumination was by means of an HBO-100 Mercury lamp, 4 mm BG 38 and 2 mm UG1 exciter filters, a TK 400 dichroic beam-splitting mirror with a K400 suppression filter, and a K430 filter in the eyepiece slot. This combination of filters produced excitation in the UV range with emissions in the green (520 nm) range and gave excellent resolution between specific and nonspecific fluorescence. A biopsy was recorded as positive only if there was definite fine or medium granular, or fibrillar fluorescence at the DEJ. Larger cytoid globules of fluorescence in the DEJ in the absence of such granular fluorescence were not considered significant since they were seen in several skin sections from a variety of normal controls and patients with other diseases.

Statistical analysis. All statistical analyses were performed on a Univac (Univac Corp., Philadelphia) 1106 Computer using the standardized SPSS programs (43). The Yates correction was applied automatically to any chi-square calculations which involved small numbers of samples.

RESULTS

Skin histology. Studies of the lesional skin in 19 of 22 biopsies from patients with SLE revealed changes typical of SLE or discoid lupus in 12 patients (acute SLE in five specimens, discoid lupus in three specimens, nonspecific vasculitis in two, subepidermal bullae in one, and basal liquefaction in one). Six other specimens showed mild or nonspecific changes (e.g. hyperkeratosis, edema without vasculitis, "acute" and "chro-

[2] Kindly supplied by Dr. I. H. Lepow.

[3] Schrager, M., J. Chapitis, I. H. Lepow, and N. F. Rothfield. Unpublished observations.

TABLE I

Protein Deposition in the DEJ of Patients with SLE

Protein	Clinically normal skin		Lesional skin		Histologically typical lesional skin	
	No. +/ No. tested	Percent +	No. +/ No. tested	Percent +	No. +/ No. tested	Percent +
IgG	14/39	46	11/22	50	6/12	50
IgM	27/39	69	14/22	64	11/12	92
IgA	7/39	18	1/22	5	1/11	9
C3	19/39	49	13/22	59	11/12	92
C4	26/39	67	11/20	55	10/12	83
Properdin	19/38	50	8/20	40	7/12	58
Any protein	33/39	85	17/22	77	11/12	92

nic" inflammation) and one specimen showed changes typical of lichen planus.

Clinically normal skin from SLE patients was studied histologically in 37 biopsies. In 34, no abnormalities other than atrophy or folliculitis were seen. However, in three specimens, the skin was found to be significantly abnormal, showing changes of acute LE in two and basal liquefaction in one. These three specimens have been included with other clinically normal appearing biopsies for the purpose of analysis. It should be noted that the two specimens of normal skin which showed histologic changes were from patients with severe skin lesions elsewhere and in both patients histologic changes in normal skin were milder than those in the lesional skin.

Skin immunofluorescence. The incidence of each protein in the DEJ is shown in Table I. A few biopsy specimens were not studied for the presence of all six proteins because of insufficient tissue. In general, deposits of IgA were less intense and less dense than other proteins. A typical example of DEJ fluorescence using antiproperdin is shown in Fig. 2. The incidence of positive DEJ immunofluorescence was similar in both normal and lesional skin. There was no statistically significant difference between normal and all lesional skin for any of the proteins studied. Of the five biopsies of lesional skin which showed no deposits of any protein, one revealed histologic changes of discoid lupus, three showed nonspecific changes, and a fourth was not examined histologically. 12 of the 22 biopsies of lesional skin revealed histologically typical abnormalities (see above) while 10 of the 22 biopsies did not reveal histologically typical abnormalities. Of the lesional skin from 12 patients with typical histologic findings of either discoid or systemic lupus, 11 or 92% had deposits of at least one protein. IgM and C3 were seen in all but one of the patients (92%), while C4 was seen in 10 (83%). There was a significantly higher incidence of deposition of IgM, C3, and C4 in the 12 histologically typical lupus lesions than in the 10 lesions which were not typical ($P < 0.05$), using Fischer's exact test. IgG was present in 50% and IgA in 8% of the histologically typical lesions, an incidence similar to that of the histologically nonspecific lesions.

Because the alternative pathway has been shown to be activated by aggregated IgA (8), we analyzed the data in relation to deposition of properdin and IgA in the same specimen. Properdin was found in 19 biopsies in which no IgA could be demonstrated, IgA was found in 4 biopsies which did not contain properdin, and in 9 biopsies both proteins were present.

The biopsy which showed histologic changes typical of atrophic lichen planus had large globules of immunofluorescence when stained for properdin and showed weak, finely granular DEJ fluorescence when stained for IgG.

Biopsies of clinically normal and lesional skin were performed simultaneously in 10 patients. There was no significant difference in individual protein deposition between lesional and normal skin except for C3 which was present in 9 of the 10 lesions and in only 3 of the 10 normal skin samples (chi-square 5.2083, $P < 0.05$). In none of the 10 patients was C3 present in normal skin without it also being found in the lesion.

None of the skin from the normal individuals stained for any protein except for the skin from the sister of the SLE patient in which properdin was present. Normal skin from the 13 control patients with other diseases was negative except in 3 patients. C4 was the only protein detected in the skin from the patient with Raynaud's disease and one patient with arthralgias. C3 alone was present in the skin from the patient with a history of cutaneous vasculitis. Weak staining was present in the DEJ from lesional skin of seven control patients with other diseases: three of seven patients with systemic sclerosis had positive tests (properdin in one, IgM, and C4 in one, and IgM, C3, and C4 in one). The latter patient was unusual in that she had

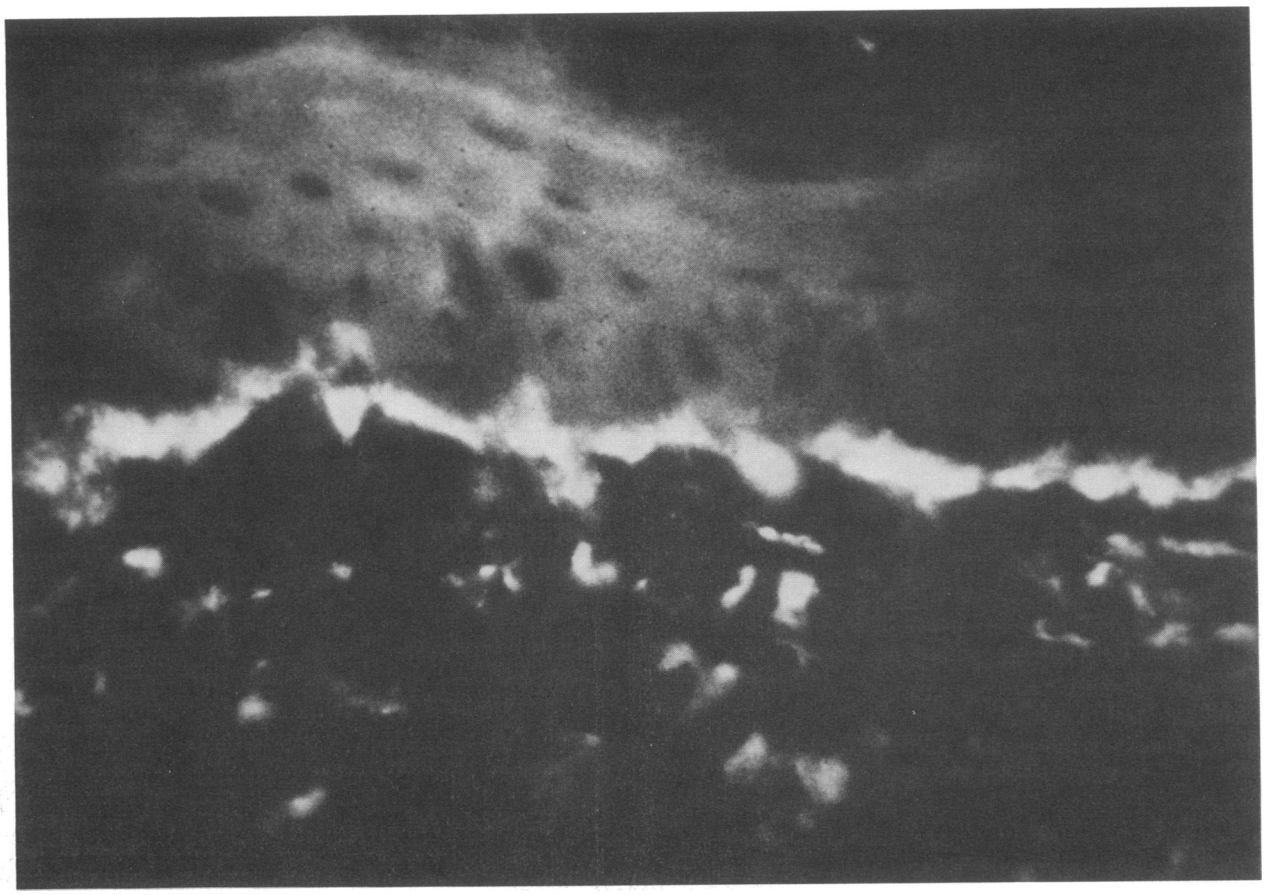

FIGURE 2 Immunofluorescence of the DEJ of normal skin from the deltoid region from a patient with active SLE stained for properdin. An intensely staining band of granular fluorescence is present at the DEJ. (Original magnification × 216).

systemic sclerosis with sclerodactyly, Raynaud's phenomena, rheumatoid nodules, latex fixation of 1:320, ANA speckled pattern with a titer of 1:256, C3 of 116 mg/100 ml, and a negative LE cell test. Two of four patients with cutaneous vasculitis were positive (C3 and properdin in one, and C3, and C4 in one). The patient with juvenile rheumatoid arthritis whose erythema multiforme-like rash was biopsied had C3 in the DEJ. The patient with rheumatoid arthritis whose lichen planus lesion was biopsied had C3 deposited in a granular pattern as well as globular deposits in the DEJ.

Complement profile. A total of 38 specimens of normal SLE skin and 19 specimens of lesional skin from SLE patients were studied for the presence of C3, C4, and properdin. For the purpose of analysis of the data, the classical pathway was considered to be involved in the skin if C4 was present with or without C3. The alternative pathway was considered to be involved if properdin was present. Both pathways were considered to be involved if both C4 and properdin were present. C3 alone was considered to be evidence for the involvement of either pathway. The results of such analysis in normal and lesional skin from SLE patients are shown in Table II. The alternative pathway was involved without involvement of the classical pathway in only two normal and two lesional skins in four patients. Although properdin was present in 50% of normal and in 40% of lesional skin, it was unusual to find properdin without also finding C4. 74% of clinically normal and 65% of lesional skin showed deposition of at least one of the three proteins (C3, C4, or properdin).

Correlation between clinical disease activity and protein deposition in DEJ. The correlation between degree of clinical disease activity and the presence of each protein in the DEJ of normal skin of SLE patients is shown in Table III. The presence of IgA was associated with disease activity: i.e., none of the 16 patients in remission had IgA in the DEJ while 4 of the 12 patients with 1+ activity and 3 of the 11 patients with 2+ to 3+ activity had IgA deposits. The higher incidence of IgA deposits in normal skin from SLE patients with

TABLE II

Complement Proteins in Normal and Lesional Skin from SLE Patients

Pathway involved	Normal skin (38 patients) No. +	Normal skin (38 patients) Percent +	Lesion skin (20 patients) No. +	Lesion skin (20 patients) Percent +
Both*	17	45	6	30
Classical‡	8	21	5	25
Alternative§	2	5	2	10
C3 alone	1	3	0	0
No C components	10	26	7	35

* C4 and properdin with or without C3.
‡ C4 with or without C3.
§ Properdin with or without C3.

disease activity was statistically significant ($P < 0.05$) by chi-square. Although there was a trend towards a higher incidence of both C3 and properdin with increasing degree of disease activity, the data did not achieve statistical significance ($0.1 > P > 0.05$).

The correlations between clinical disease activity and protein deposition in lesional skin from SLE patients are shown in Table IV. Since all patients with active skin lesions showed, by definition, at least 1+ activity, the comparison was made between those with only skin manifestation (1+ active) and those with additional extradermal manifestations of activity (2–3+). Each protein was found more frequently in the skin lesions of patients with additional extradermal evidence of activity than in skin lesions of patients whose only evidence of clinical disease activity at the time of biopsy was skin lesions. This correlation with disease activity was statistically significant only for the deposition of properdin ($P < 0.02$). Patients with renal disease (14 patients) or those with hematologic manifestations (8 patients) were found to have no significant difference in frequency of protein deposition from those patients without these manifestations. The presence of skin lesions did not apppear to affect the frequency of deposits in normal skin since there was not a higher frequency of protein deposition in normal skin from patients with skin lesions than in those without skin lesions. Thus, the frequency of protein deposition correlates with general disease activity rather than with any specific manifestation of the disease.

TABLE III

Correlation between Protein Deposition in the DEJ of Normal Skin from SLE Patients and Clinical Disease Activity

Protein	Clinical disease activity, *Percent* + 0 (16 patients)	1+ (12 patients)	2+ or 3+ (11 patients)
IgG	25	42	46*
IgM	56	75	82*
IgA	0	33	27‡
C3	38	42	73*
C4	63	58	82*
Properdin	33	50	73*

* Not significant ($P > 0.05$) by chi-square.
‡ $P < 0.05$ by chi-square.

TABLE IV

Correlation between Protein Deposition in the DEJ of Lesional Skin from SLE Patients and Clinical Disease Activity

Protein	Clinical disease activity, *Percent* + 1+ (14 patients)	2+ or 3+ (8 patients)
IgG	43	63*
IgM	50	88*
IgA	0	13*
C3	43	88*
C4	50	67 (6 cases)‡
Properdin	21	83 (6 cases)§

* Not significant ($P > 0.05$) by chi-square with Yates correction.
‡ Not significant ($P > 0.05$) by Fisher's exact test.
§ $P < 0.02$ by Fisher's exact test.

Serologic studies. The results of tests for antinuclear antibody titers, DNA-binding, C3, C4, and properdin in the sera of 51 SLE patients and 49 healthy individuals are shown in Table V. The SLE patients showed abnormalities for all tests performed except that the

TABLE V

Serological Parameters in SLE Patients and Controls

Serological test	SLE patients Mean±1 SD (range) (51)‡	Normal individuals Mean±2 SD (range) (48)‡
ANA titer (*n*)*	6.0±2.6 (0–11)	3.7±2.1 (0–5)
DNA binding, %	43±21 (0–100)	16±23 (0–55)
C3, *mg/100 ml*	64±22 (22–114)	80±28 (48–112)
C4, *mg/100 ml*	17±11 (1–55)	34±24 (17–70)
Properdin, (*% of normal pool*)	107±24 (40–157)	99±34 (47–125)

* *n* is the $-\log_2$ of the geometric mean titer of antinuclear antibody. Only patients with a positive ANA test are included in the calculation. 21 of the normal individuals and 2 of the SLE patients had negative ANA at the time of study.
‡ Number of determinations.

TABLE VI

Correlation between Serological Parameters and Clinical Disease Activity

Serologic test	Clinical disease activity			Significance‡
	0 (16 patients)	1+ (19 patients)	2+ or 3+ (15 patients)	
ANA Titer (*n*)	4.9±3.1*	5.8±2.5	7.3±1.2	$P < 0.05$
DNA binding, %	24±16	33±27	61±32	$P < 0.01$
C3, *mg/100 ml*	80±22	64±16	43±15	$P < 0.01$
C4, *mg/100 ml*	25±13	14±7	10±8	$P < 0.01$
Properdin, *% of normal pool*	113±21	112±24	92±22	$P < 0.05$

* Mean±1 SD.

‡ Analysis of variance.

mean properdin level for the entire group of SLE patients was normal. The relation between the serologic parameters and clinical disease activity is shown in Table VI. Antinuclear antibody titer and DNA binding both correlated positively with clinical disease activity, while C3, C4, and properdin levels correlated negatively with disease activity. Correlation with disease activity was strongest for C3, followed in order by C4, DNA binding, properdin, and antinuclear antibody titer.

The correlation between each pair of serologic parameters was calculated using regression analysis. ANA titers had a stronger negative correlation with C4 levels ($r = -0.51$, $P = 1.3 \times 10^{-4}$) than with properdin levels ($r = -0.2$, $P = 6.5 \times 10^{-2}$). Similarly, DNA binding had a stronger negative correlation with C4 levels ($r = -0.48$, $P = 4.8 \times 10^{-4}$) than with properdin levels ($r = -0.43$, $P = 1.04 \times 10^{-3}$). C4 levels correlated strongly with C3 levels ($r = 0.67$, $P = 1 \times 10^{-6}$) and poorly with properdin levels ($r = 0.37$, $P = 5.6 \times 10^{-3}$).

Correlation between serologic parameters and protein deposition. The relationship between the deposition of C3, C4, or properdin in the DEJ and the serum level of that particular protein was evaluated by analysis of variance. The deposition of a specific protein correlated with depression of the serum level of that protein in only one instance: properdin in lesional skin. The mean (±SD) serum properdin level in the 12 patients in whom properdin was not demonstrated in lesional skin was 119±22% while that in the 8 patients with properdin in the lesional skin was 83±22% ($P < 0.05$). Although serum C3 levels were lower in patients with C3 skin deposits in both normal and lesional skin than in those without C3, the difference was not statistically significant.

The data were analyzed to determine if the presence of skin lesions was associated with changes in the various serologic parameters. There was no significant difference in DNA binding (mean±1SD) in sera from patients with or without skin lesions. Similarly the mean properdin level in patients without skin lesions was not significantly different from that in patients with skin lesions. On the other hand, the mean C3 level was significantly lower in patients with skin lesions (57 ±18 mg/100 ml) than in those without skin lesions (72±25 mg/100 ml) ($P < 0.05$) and the mean C4 level was significantly lower in patients with skin lesions (13±8 mg/100 ml) than in patients without skin lesions (22±13 mg/100 ml ($P < 0.01$).

DISCUSSION

In this study we have shown several significant correlations between clinical disease activity, serologic abnormalities, and dermal immunopathology in SLE patients.

We have shown that there is a significant correlation between clinical disease activity and the presence of IgA in the DEJ of nonlesional skin. Others have correlated a positive lupus band test with disease activity (21, 22, 24) and we now document an association between deposition of a specific protein and disease activity. Our data revealed that IgA was absent in all nonlesional skin from patients with no evidence of clinical disease activity, while it was present in 7 of 23 with active disease. A higher but not statistically significant increase in incidence of IgG, IgM, and complement proteins was also found in normal skin from patients with clinically active disease.

We were unable to correlate the presence of any single immunoglobulin or complement protein with the presence of clinical disease activity in any specific organ system. The association between the presence of these proteins in the DEJ and lupus nephritis has been suggested by Burnham and Fine (21) and subsequently by Gilliam et al. (22). Caperton et al. in evaluating a population with a higher incidence of lupus nephritis (23 of 29 patients), found no such correlation (23). Some of the differences noted in incidence of deposition of proteins may be related to the sites biopsied. Immunoglobulins have been generally found in a much lower incidence (35–55%) in biopsies taken from

the flexor surface of the forearm (21, 22, 44–46). Since we used the deltoid area for biopsies of normal skin, the high incidence of deposits found in the SLE patients without clinical evidence of renal disease may be due to our choice of the site of biopsy. Because of the high incidence of deposition of proteins in nonlesional skin from our patients without clinical evidence of renal disease, we cannot conclude that studies of nonlesional skin taken from sun-exposed areas are of value in assessing the presence of renal disease in SLE. Our patients have been followed for 8 mo–4 yr after skin biopsy and none of the patients without clinical renal disease at the time of biopsy have developed renal disease. Thus, although the deposition of proteins, especially IgA, in nonlesional skin from the deltoid region reflects clinical disease activity at the time of biopsy, no correlation with renal disease can be made. Further long-term serial studies on individual patients during disease activity and remission are needed to confirm this observation.

We have shown that properdin was deposited in biopsies of nonlesional skin from 50% of patients with SLE. This is a somewhat higher incidence than that reported by Provost and Tomasi (27) who found properdin in 5 of 25 biopsies, or by Jordon et al. (28) who found it in 2 of 9 biopsies of normal skin from SLE patients. In our series, properdin was present in 5 of 15 biopsies from nonlesional skin of patients in remission and in 8 of 11 from patients with active disease. Although not statistically significant, the data suggest a correlation between disease activity and properdin deposition in nonlesional skin.

We have previously reported the presence of properdin in lesional skin from SLE patients (26). In the present study, properdin was found in 8 of 20 biopsies of skin lesions from SLE patients. It is of interest that the 12 biopsies of clinically abnormal skin which showed histologic changes typical of SLE revealed properdin in 7.

These data suggest that the alternative pathway is involved in tissue damage in SLE. The serologic data support this hypothesis. Serum properdin levels from patients with clinical disease activity were significantly lower than levels from patients in remission. In addition, patients with properdin in their skin lesions had a significantly lower serum properdin level than patients without properdin deposition. These findings suggest that properdin is being more rapidly consumed in patients during periods of nonrenal clinical disease activity. Increased catabolism of properdin has been directly demonstrated in patients with active lupus nephritis (33), but further turnover studies are necessary to confirm our findings in patients without nephritis.

While the evidence for the involvement of a protein from the alternative pathway was present in both lesional and nonlesional biopsies, the classical pathway was clearly involved in the majority of patients. Proteins from the classical pathway were demonstrated in the majority of skin biopsies of both normal and lesional skin. The serologic data also support the conclusion that the classical pathway is involved. Thus, C4 levels were significantly lower in patients with disease activity at the time of biopsy than in those in remission. In addition, DNA binding and titer of antinuclear antibodies also correlated with the presence of disease activity.

We have studied the relationship between proteins of the alternative and classical pathways by regression analysis of the serologic data. We have shown that the strongest association exists between serum levels of C3 and C4 suggesting primary activation of the classical pathway. The involvement of the classical pathway was also suggested by the association between elevated DNA binding and ANA titer with C4. Additional involvement of the alternative pathway is suggested by the association between properdin and C3. Thus, the data suggest that both pathways are activated in patients with clinically active SLE.

In previous serologic studies neither Perrin et al. (30) nor McLean and Michael (31) were able to show a correlation between serum properdin levels and serum C3 levels. These investigators limited their patients to those with lupus nephritis and studies of DNA antibodies or antinuclear antibodies were not reported. Although no attempt was made to correlate their findings with disease activity, McLean and Michael described a significant decrease in properdin levels in SLE patients before immunosuppressive therapy. Perrin et al. (30) were unable to show a positive correlation between C3 levels and properdin, but they did find significant depression of properdin in those patients with low C3 levels as compared to normal controls. No analysis of antinuclear antibodies was included in their studies. Additional evidence for involvement of proteins of the alternative pathway in SLE patients with nephritis has been reported by these authors and others, (32, 47) in studies of factor B.

Recent studies by Chapitis and Lepow (48) and Fearon and Austen (17) indicate that properdin binds to native C3, C3b, or C3c in free solution, or to red cells coated with complement (EAC 43B). Thus, the possible consumption or binding of properdin via C3 was investigated in our patients. Properdin and C3 were present together in normal skin from 12 patients; properdin was detected alone in 1, and C3 alone in 7. Both proteins were present in lesional skin from 7 patients; properdin alone in 1, and C3 alone in 4. Thus,

in 19 biopsies either properdin or C3 was demonstrated in the absence of the other. We would therefore conclude that such binding of properdin to C3 is not regularly detectable in SLE skin.

The activation of the classical pathway in SLE may be attributed to the immune complexes demonstrable in the serum of patients with this disease. The mechanism for activation of the alternative pathway is unknown in SLE. The possible presence in the sera of patients of substances similar to complex microbial polysaccharides cannot be excluded. Aggregated IgA has also been shown to activate the alternative pathway in vitro, and Evans et al. (49) have described four patients with Henoch-Schonlein purpura or focal nephritis in whom only IgA, properdin, and C3 could be demonstrated in mesangium of kidney biopsies. No such distribution of these three proteins was demonstrated in the skin of our patients, and properdin was present in a much greater number of biopsies than IgA. The alternative pathway can be activated by C3NeF which has been demonstrated in sera from patients with chronic glomerulonephritis. A similar factor has not yet been observed in sera from patients with SLE.

ACKNOWLEDGMENTS

We wish to thank Dr. Peter B. Hukill, Department of Pathology, for his reading of the light microscopy of the skin biopsies. We also thank Ms. Bonnie Bordwell, Ms. Carol Manning, and Mrs. Loretta Albert for their excellent technical assistance, and Mrs. Marilyn Cowles and Mrs. Susan Crawford for preparation of the manuscript.

This work was supported by research grant AM 16576, a grant from the Connecticut Lupus Foundation and an Arthritis Foundation Clinical Research Center Grant.

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