Antibodies to T- and L-Isoforms of the Cytoskeletal Protein, Fimbrin, in Patients with Systemic Lupus Erythematosus

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Abstract

The cytoskeleton is a complex network of proteins that maintain cell shape, mobility, and organelle function. Its components can be divided into three distinct classes: microfilaments, microtubules, and intermediate filaments. Fimbrins are microfilament proteins, a family of cytoplasmic phosphoproteins. Expression of the L-fimbrin isoform is restricted to replicating blood cells and expression of the T-fimbrin isoform to replicating cells of solid tissues. Sera from normals and from patients with systemic lupus erythematosus (SLE), juvenile arthritis, rheumatoid arthritis, Sjögren's syndrome, osteoarthritis, vasculitis, scleroderma, and mixed connective tissue disease were tested for the presence of antibodies to T- and L-fimbrin by ELISA, using purified recombinant fimbrin. The mean OD of sera from SLE patients was significantly higher than in normals (T-fimbrin, $P < 0.0001$; L-fimbrin, $P < 0.001$). 48 of 98 SLE sera had antibodies to T-fimbrin; 32 had antibodies to L-fimbrin; 20 had antibodies to both; 28 had only anti-T, and 12 had only anti-L-fimbrin. The mean OD for sera of the other rheumatic diseases was not significantly different from normals. The presence of either L- or T-fimbrin antibody was associated with pleuropericarditis ($P = 0.015$), photosensitivity ($P = 0.011$), and anti-Sm antibody ($P = 0.010$). Central nervous system SLE was associated with the presence of the L-fimbrin antibody alone ($P = 0.016$). There was a strong association between DR7 (but not other MHC alleles) and anti-L-fimbrin antibodies in SLE patients (chi square = 18; $P < 0.00002$). No MHC association was observed with anti-T-fimbrin antibodies. (*J. Clin. Invest.* 1992. 90:1037–1042.) Key words: enzyme-linked immunosorbent assay • major histocompatibility complex • systemic lupus erythrematosus

Introduction

The cytoskeleton is a complex network of protein filaments that maintain cell shape, mobility, and organelle function (1, 2). Three distinct classes of cytoskeletal filaments are organized in the cytomatrix: (*a*) microfilaments that are actin-containing fibers; (*b*) microtubules, hollow fibers composed primarily of tubulin (3); and (*c*) intermediate filaments consisting of at least five biochemically and immunologically distinct types; each filamentous protein is associated with a range of accessory proteins that serve to stabilize filments, bridge adjacent filaments, and link filaments to organelles or to the plasma membrane (4). The major protein subunit of microfilaments is actin. Actin filaments are commonly organized in the cytoplasm into bundles or gels formed by separate classes of actin cross-linking proteins (5).

The human L- and T-fimbrins are (70–72%) homologous to chicken fimbrin, which is an f-actin bundling protein found in intestinal microvilli, hair cell stereocilia, and fibroblast filopodia (6). L-fimbrin is expressed in the cytoplasm of leukocytes (7); T-fimbrin is constitutively expressed in epithelial and mesenchymal cells derived from solid tissues, but neoplastic cells from these tissues express both fimbrin isoforms (6). Fimbrin is composed of two structural domains, a headpiece (amino terminus) and a core (carboxy terminus), linked by a protease sensitive region. Sequence homologies with other actin- or calcium-binding proteins suggest that these domains have separate functional roles: the headpiece binds calcium and the core binds actin (6). The pattern of fimbrin isoform expression may also be correlated with mobile or adhesive properties of the cell, because specific antibodies have detected fimbrin in focal contact and adhesion sites on the ventral surface of cultured cells, monocytes, and macrophages (8).

Cytoskeleton proteins have been shown to be a target for an autoimmune reaction in systemic lupus erythematosus (SLE)[1] (9–13). Sera from patients with SLE and related disorders were tested for the presence of anti-L- and anti-T-fimbrin antibodies using an ELISA and through chart analysis, which sought to determine antibody clinical and laboratory correlates. We also examined HLA phenotypes to determine if genetic factors, encoded within the MHC, were associated with the presence or absence of antibodies to L- and T-fimbrin in the sera of SLE patients.

Methods

Patients. 98 sera were obtained from patients with established SLE according to the ACR criteria (14), 12 with RA, 12 with Sjögren's syndrome (SS), 12 with scleroderma, 10 with systemic vasculitis (SV), 12 with juvenile rheumatoid arthritis (JRA), 13 with osteoarthritis (OA), 3 with mixed connective tissue disease (MCTD), and 59 normals as described previously (15). Sera of patients with rheumatic diseases included in this study were diagnosed according to established criteria (15). All sera were stored at −80°C until used.

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Received for publication 21 May 1991 and in revised form 20 March 1992.

J. Clin. Invest.

0021-9738/92/09/1037/06 $2.00
Volume 90, September 1992, 1037–1042

1. *Abbreviations used in this paper:* ANA, antinuclear antibodies; JRA, juvenile rheumatoid arthritis; MCTD, mixed connective tissue disease; OA, osteoarthritis; SS, Sjögren's syndrome; SV, systemic vasculitis.

Antinuclear antibodies and genetic typing. Antinuclear antibodies (ANA) were detected by immunofluorescence on mouse liver and HEP-2 cells. All sera positive for ANA were also examined by counterimmunoelectrophoresis for antibodies to ssDNA, Sm, RNP, Ro, La, SCL-70, and PCNA (16). Antibodies to dsDNA and cardiolipin were detected by ELISA (15). HLA and complement allotyping of SLE patients were performed as described previously (17).

ELISA. An ELISA for detection of antibodies to the T- and L-isoforms of fimbrin was developed based on methods previously described (15), with some modifications. Polystyrene microtiter plates (Costar, Cambridge, MA) were coated overnight with 100 μg of human recombinant T- or L-fimbrin (isolated to homogeneity as described previously [6]) in 100 μl of 50 mM carbonate-bicarbonate buffer, pH 9.6. On the following day, the excess fluid was removed and free sites were blocked for 2 h at room temperature with 250 μl of 5% skimmed milk in 10 mM sodium phosphate, pH 7.2, 150 mM sodium chloride, 0.05% Tween 20 (PBS-Tween). The plates were then washed three times with PBS-Tween. 100 μl of sera (1:100 diluted in 1% Carnation® skimmed milk in PBS-Tween) were incubated in each coated well for 2 h at 37°C. Each serum sample was tested in duplicate. Plates were washed three times with PBS-Tween to remove the unbound material. 100 μl of alkaline phosphatase–conjugated goat anti-human IgG (Sigma Chemical Co., St. Louis, MO), diluted 1:1,000 in 1% skimmed milk in PBS-Tween was added and incubated for 2 h at 37°C. The plates were washed three times with PBS-Tween and 100 μl of 1 mg/ml *p*-nitrophenyl phosphate added. Plates were read at 405 nm using a multiscan ELISA reader (Titertek, Elfab Oy, Finland) and the results were expressed as OD.

Inhibition assay. Four SLE sera positive for antibodies against T- or L-fimbrin were diluted 1:100 and mixed with equal volumes of 100 μg to 1 mg T- or L-fimbrin. The samples were incubated overnight at 4°C before being assayed in the ELISA for antibodies to T- and L-fimbrin.

Western blots. Western blot analysis was performed as previously described (18) with some modifications. 25 μg of purified T- or L-fimbrin was subjected to electrophoresis on 10% SDS-polyacrylamide gel. Proteins were electrophoretically transferred to nitrocellulose membrane (Schleicher & Schuell, Keene, NH) by using the Trans-blot cell apparatus (Bio-Rad Laboratories, Richmond, CA) overnight at 4°C, at a current of 200 mA, in Tris–glycine (25 mM Tris, 192 mM glycine) buffer containing 20% methanol. Proteins were used as molecular-weight markers (Bio-Rad Laboratories). Protein blot strips were incubated with 5% skimmed milk in PBS-Tween for 2 h at room temperature to block the unreacted membrane sites. The blot strips were subsequently incubated with sera diluted 1:100 in 1% skimmed milk with PBS-Tween at room temperature. After 1 h, the blots were washed three times with PBS-Tween and incubated for 1 h with alkaline phosphatase–conjugated goat anti-human IgG (Sigma Chemical Co.) diluted 1:1,000 in 1% skimmed milk in PBS-Tween at room temperature. The strips were again washed three times with PBS-Tween and developed by using AP color reagent (Bio-Rad Laboratories) containing premixed nitrobluetetra-zolium and 5-bromo-4-chloro-3-indoxyl phosphate. The reaction was stopped after 20 min by transferring the strips to distilled water for 2 min.

Charts from the 98 patients with SLE were reviewed for clinical and laboratory features. These included a history of arthritis, arthralgias, discoid and butterfly rashes, photosensitivity, mouth ulcers, pleuropericarditis, miscarriage, central nervous system signs and symptoms (seizures, psychiatric disturbances, strokes), renal disease, Raynaud's phenomenon, pulmonary disease (restrictive or interstitial lung disease), or livedo reticularis (Table I). Laboratory features were abstracted from the patient's charts on the serum sample date as well as over the patient's recorded history. These features were defined as follows: thrombocytopenia (a platelet count < 150,000) proteinuria (1+ or more on dipstick examination), and leukopenia (white blood cell count < 3,500/mm^3). Absolute values for the lowest values of total complement (CH50), lowest hematocrit, and highest creatinine were abstracted as well. Charts were also reviewed for the presence of ANAs and other autoantibodies (Table II). Patients were classified as having a diagnosis of SLE if they met ≥ 4 of the 11 criteria for the disease (14).

*Table I. Clinical Features in 81 Patients with Antibodies to L- and/or T-Fimbrin**

Signs and symptoms	Fimbrin (L or T) (n = 49)	L-fimbrin (n = 24)	T-fimbrin (n = 42)
Arthritis (n = 80)	42	22	36
Arthralgias (n = 80)	45	22	39
Butterfly rash	44	22	38
Discoid rash (n = 77)	12	6	11
Photosensitivity (n = 80)	47‡	24§	40‖
Mouth ulcers (n = 80)	8	4	7
Miscarriage history (n = 76)	3	2	3
Livedo reticularis (n = 65)	7	3	5
Pulmonary disease (n = 77)	5	1	5
Renal disease (n = 74)	25	14	22
Central nervous system manifestations (n = 79)	14	10¶	12
Psychiatric disease (n = 76)	8	6	7
Raynaud's (n = 78)	35	15	31
Pleuropericarditis (n = 79)	24**	12	20

* n = 81 unless otherwise noted. ‡ P = 0.011 versus no antifimbrin. § P = 0.030 versus no anti-L-fimbrin. ‖ P = 0.040 versus no anti-T-fimbrin. ¶ P = 0.016 versus no anti-L-fimbrin. ** P = 0.015 versus no antifimbrin.

Statistical methods. The history of clinical laboratory and autoantibody features was recorded as dichotomous variables and analyzed with the SAS statistical package (19). Tests of association among variables were done with a chi-square statistic or Fisher's Exact Test. Univariate analysis between continuous variables was done with a t test

*Table II. Laboratory Features in 81 Patients with Antibodies to L- and/or T-Fimbrin**

Feature	Antifimbrin (L or T) (n = 49)	Anti-L-fimbrin (n = 24)	Anti-T-fimbrin (n = 42)
Thrombocytopenia (n = 71)	4	2	3
Leukopenia (n = 73)	12	6	9
Proteinuria (n = 69)	18	8	15
Lowest CH50 mean (SD)	192.3 (72.6)	183.8 (70.7)	196.8 (74.8)
Lowest hematocrit mean (SD)	37.5 (4.8)	37.8 (4.4)	37 (4.9)
Highest creatinine mean (SD)	1.3 (0.9)	1.3 (1.9)	1.4 (0.9)
Anti-dsDNA (n = 72)	32‡	18	26
Anti-ssDNA (n = 71)	22	13	18
Anti-RNP (n = 71)	23	11	19
Anticardiolipin (n = 34)	12	5	10
Anti-Ro (n = 65)	19	9	15
Anti-La (n = 65)	10	6	8
Anti-Sm (n = 72)	23§	13	20‖

* n = 81 unless otherwise noted. ‡ P = 0.040 versus no antifimbrin. § P = 0.010 versus no antifimbrin. ‖ P = 0.025 versus no anti-T-fimbrin.

statistic. A significance level of 0.05 was used for each test. No adjustments were made for multiple testing.

Results

SDS-PAGE and Western blot

The purity and homogenity of the recombinant T- and L-fimbrin preparation was confirmed by SDS-PAGE. 25 μg of T- or L-fimbrin subjected to SDS-PAGE were transblotted onto nitrocellulose sheets and probed with human sera containing anti-T- or L-fimbrin-specific antibodies. Major species identified for T- and L-fimbrin were 70 and 68 kD, respectively, in SDS-PAGE (Fig. 1 *A*) as well as in Western blot analysis (Fig. 1 *B*).

Detection of anti-T- or anti-L-fimbrin antibodies by ELISA

T-fimbrin. 59 normal human sera were tested for presence of anti-T-fimbrin antibodies; the mean OD was 0.201±0.132 (1 SD). An OD of 0.597 (mean±3 SD) was assigned as the upper limit of normality. Henceforth any sera with an OD over the upper limit of normal was considered as "positive;" sera with OD within the normal range were considered as "negative." 1 of 59 (1.7%) normal sera and 48 of 98 SLE sera (49%) had anti-T-fimbrin antibodies. One of 12 (8.3%) SS sera and 1 of 10 (10%) SV sera were also positive. Sera of patients with RA, JRA, OA, scleroderma, and MCTD were all negative (Fig. 2).

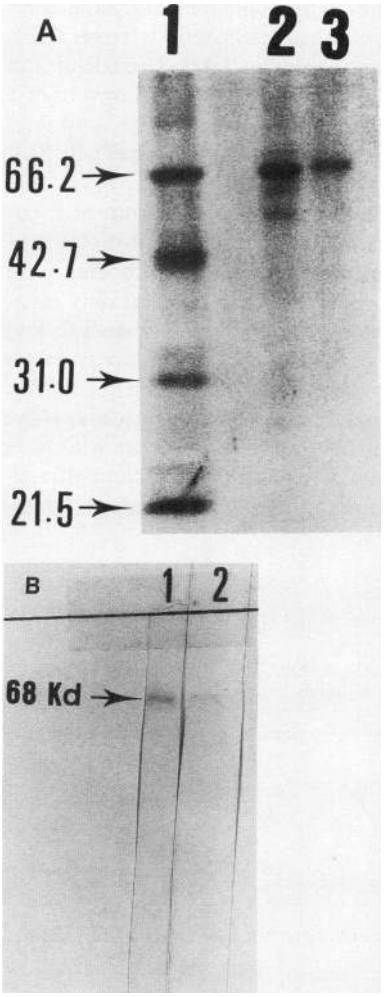

Figure 1. (*A*) Analysis of purified recombinant L-fimbrin (lane *2*) and T-fimbrin (lane *3*) by SDS-PAGE. Lane *1* has molecular weight markers (BSA, 66.2 kD; ovalbumin, 42.7 kD; carbonic anhydrase, 31 kD; and trypsin inhibitor, 21.5 kD). Electrophoresis was performed as indicated in Methods. (*B*) Western blot analysis of L-fimbrin and T-fimbrin using human antisera against L-fimbrin (lane *1*) and T-fimbrin (lane *2*) as described in Methods. The molecular weight of the antigens recognized by the antisera is marked.

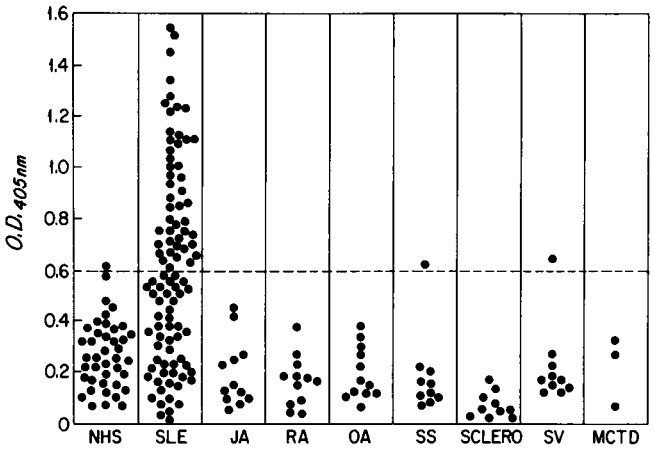

Figure 2. Anti-T-fimbrin antibody profile in normal human sera (*NHS*) and sera of patients with various connective tissue diseases. Antibody detection was carried out by ELISA as described in Methods. Some of the NHS gave identical OD and their circles are superimposed on each other.

The mean OD of sera from SLE patients, 0.613±0.369 (1 SD), was significantly higher than the mean OD of normal sera ($t = -8.26$; $P < 0.0001$). The mean OD for sera of patients with other rheumatic diseases was not significantly different from the mean OD for normal sera.

L-fimbrin. The mean OD of 55 normal human sera was 0.138±0.055 (1 SD). An OD of 0.303 (mean±3 SD) was taken as the upper limit of normal (Fig. 3). By this criterion, 32 of 98 (32.6%) SLE sera tested were positive. Sera from normals and patients with MCTD and SV were negative. 1 of 12 JRA (8.3%), 1 of 12 RA (8.3%), 1 of 12 OA (8.3%), 3 of 12 SS (25%), and 1 of 12 scleroderma sera (8.3%) were also positive. SLE patients had a mean OD of 0.280±0.055 (1 SD), which was significantly higher than OD of normal sera ($t = -4.221$; $P < 0.001$).

Inhibition by T- and L-Fimbrin

Approximately 50 μg/ml of L- or T-fimbrin was sufficient to give 50% antigen specific inhibition of respective antibodies.

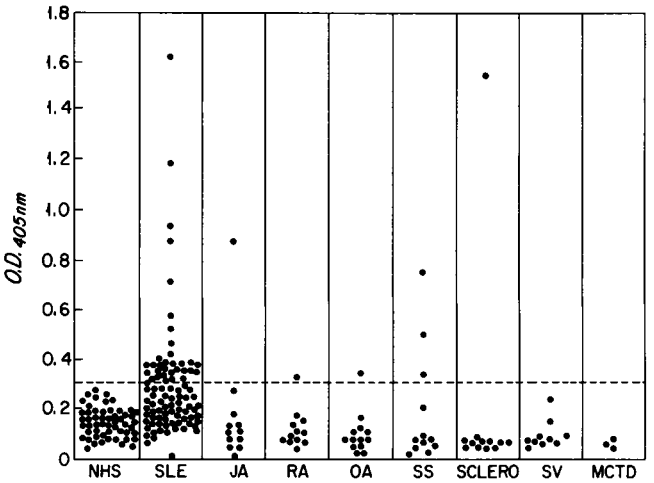

Figure 3. Anti-L-fimbrin antibody profile in normal human sera (*NHS*) and sera of patients with various connective tissue diseases. Antibodies were detected by ELISA as described in Methods.

Upon incubation of anti-L-fimbrin–positive antisera with 100 ng of T-fimbrin, only 20% inhibition was observed. 100 ng of L-fimbrin inhibited anti-T-fimbrin antibodies by only 13% (Fig. 4).

Coexistence of anti-T- and anti-L-fimbrin antibodies

48 of 98 SLE sera had antibodies to T-fimbrin; 32 had antibodies to L-fimbrin; 20 of 48 sera positive for anti-T-fimbrin had antibodies to L-fimbrin also. 20 of 32 sera positive for L-fimbrin had antibodies to T-fimbrin also.

Clinical and serological associations

81 charts were available and were reviewed for clinical correlates of the L- and T-fimbrin antibodies. 80 patients were Caucasian, 4 were male and 77 were female. No male patient had the L-fimbrin, only one had an antibody to T-fimbrin. Of the 77 female SLE patients, 7 had only anti-L-, 24 had only anti-T-, and 17 had both anti-T- and anti-L-fimbrin antibodies.

There was a positive association between the presence of the antifimbrin antibody and a history of pleuropericarditis (chi-square = 5.939, $P = 0.015$) and a history of photosensitivity (Fisher's Exact Test; $P = 0.011$) (Table I). Patients with the antifimbrin antibody were more likely to have a history of anti-Sm antibody (chi-square = 6.72, $P = 0.010$) (Table II).

There were no differences in the frequency of thrombocytopenia, leukopenia, or proteinuria in those with or without the antifimbrin antibody. Nor were there any differences in the absolute values of the CH50, hematocrit, creatinine, or the presence of other autoantibodies over time between groups.

When our analysis for clinical and laboratory correlates was stratified by L- and T-isoforms of the antifimbrin antibody, a history of central nervous system SLE (exclusive of psychiatric disorders) was found more frequently in those with the L-fimbrin antibody (chi-square = 5.857, $P = 0.016$). Psychiatric disorders appeared more frequently in those with the L-fimbrin antibody than in those without, 25 versus 9%; however, this difference did not reach significance (chi-square = 3.59, $P = 0.058$). Sm antibody was found more frequently in those with the L-fimbrin antibody (55%) versus those without L-fimbrin antibody (32%) (P = NS); T-fimbrin antibody was positively associated with Sm antibody (chi-square = 5.04, $P = 0.025$). No other clinical, laboratory, or autoantibody features were found to be associated with the L or T isoforms of the antifimbrin antibody.

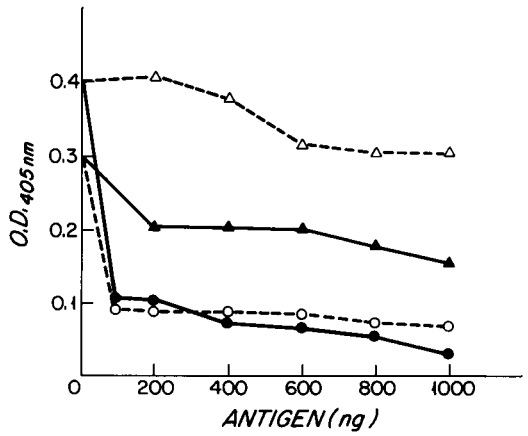

Figure 4. Inhibition assay of anti-T- and anti-L-fimbrin antibodies. ELISA plates were coated with L-fimbrin and anti-L serum was incubated with T-fimbrin (--- △ ---) and L-fimbrin (— ● —). Similarly, plates were coated with T-fimbrin and anti-T serum was incubated with L-fimbrin (— △ —) and T-fimbrin (— ○ —). The sera incubated with antigen were used to perform ELISA as described. Sera used in this assay had either anti-T- or anti-L-fimbrin antibodies, but not both.

Association with MHC antigens

57 SLE patients were typed for HLA-A,B,DR, and C4A and C4B alleles (17). No association with HLA-A, HLA-B, C4A, or C4B alleles was observed with presence or absence of antifimbrin antibodies (data not shown). A strong association between DR7 and the presence of anti-L-fimbrin antibodies was observed (Table III) (chi-square = 18.0; $P < 0.00002$). No HLA-DR association was demonstrated with anti-T-fimbrin antibodies.

Discussion

Several reports of anticytoskeletal antibodies in sera from normals (13) and from patients with connective tissue diseases (20–24) and infections (25–27) have appeared in the literature over the past decade. Antibodies to smooth muscle, now known to bind actin and desmin, have been found in sera from patients with chronic active hepatitis (28). Antibodies to a mitotic spindle occur occasionally in patients with connective tissue diseases (29). Antibodies to microfilament anchorage sites were reported in patients with early systemic sclerosis (22). Antibodies to neurofilaments occur in Jakob-Creuzfeldt and Kuru disease and SLE (12). Vimentin has been shown to be a target for an autoimmune reaction in parasitic (25) and viral infections (30), but also in rheumatic diseases, especially SLE (31, 32).

We have identified autoantibodies in sera from SLE patients that are directed against the microfilament proteins L- and T-fimbrin. Anti-L and T-fimbrin autoantibodies were found predominantly in sera of SLE patients and only occasionally in sera from patients with other rheumatic diseases and normals. SLE patients can be divided into four groups based on the presence of autoantibodies for the two fimbrin isoforms: patients who have antibodies against L-fimbrin only, those who have antibodies against T-fimbrin only, those who have antibodies to both, and those who have neither. Autoantibodies appear to be specific against either L- and T-fimbrin. Inhibi-

*Table III. HLA-DR Association of Antifimbrin Antibodies in 57 Systemic Lupus Erythematosus Patients**

	Anti-L-fimbrin antibodies		Anti-T-fimbrin antibodies	
	Positive‡	Negative	Positive	Negative
DR7 patients (n = 23)	17	6	15	8
Non-DR7 patients (n = 34)	6	28	17	17

* HLA data for remaining patients were not available. ‡ Chi-square; 18.0; $P > 0.00002$.

tion assays demonstrated only minor cross-reactivity between L- and T-fimbrin.

The heterogeneity of the L- and T-fimbrin antigen and its autoimmune response may operate in a similar way to the Ro/SSA antigen, which has (at least) four antigenically distinct isoforms (33). It is clear that there are antigenic differences between the 70 kD T-fimbrin and 68 kD L-fimbrin, as some SLE sera reacted with only one form, although in inhibition studies there was also some cross-reactivity. Final definition of these antigenic differences at the structural level will depend on characterizing the epitopes of each isoform.

A significant association between antivimentin antibodies, anticardiolipin, and anti-DNA antibodies has been reported in SLE patients (10, 34, 35). High titers of antineurofilament antibodies have occurred in SLE patients with neuropsychiatric manifestations as demonstrated by different studies (12, 13).

Our study set out to determine the clinical, laboratory, and autoantibody features associated with the antifimbrin antibody by chart review in 81 SLE patients. Few clinical differences were found between those with and without the antifimbrin antibody, exclusive of pleuropericarditis and photosensitivity and central nervous system SLE in patients with the L-isoform alone. It is of interest that our analysis demonstrated the presence of the antifimbrin antibody more frequently in those with the anti-Sm antibody. This phenomenon may be genetically influenced by the more common HLA-DR7 in this subset of SLE patients.

Specific autoantibodies have been demonstrated to be associated with a number of HLA antigens in SLE patients. DNA autoantibodies have been associated with DR3 (36), DR7 (37), and DR2 (38); antibodies to Sm have been associated with DR7 (39); antibodies to La (SS-B) have been associated with DR3 (40); and antibodies to Ro(SS-A) with the haplotype B8, DR3, SC01 (unpublished data), DR3 (38), and DR2 especially with deficiency of the second complement component (41).

No MHC associations with anticytoskeletal autoantibodies have been reported previously. We have found a strong association between DR7 (but no other MHC alleles) and anti-L-fimbrin autoantibodies in SLE patients. No MHC association was observed with anti-T-fimbrin antibodies. Further elucidation of the relationship between anti-L- and anti-T-fimbrin autoantibodies production and these genetic factors needs to be conducted.

SLE is a pleomorphic disease. Certain antibody systems found in SLE coincide with clinical features. Such laboratory clinical correlations permit the classification of the patients into subsets based upon serum antibodies (34, 41).

The description of autoantibodies against fimbrins in SLE patients raises speculations about the possible role of these autoantibodies in the pathogenesis of lupus. As previously suggested for antibodies against vimentin and cytokeratin (18), the stimulus for production of L- and T-fimbrin autoantibodies may be derived from the destruction of fimbrin-containing cells, such as replicating white blood cells (L-fimbrin) or epithelial and mesenchymal surfaces (T-fimbrin). It remains to be determined if antibodies for L- and T-fimbrins contribute to the loss of cell function or other aspects of lupus. Further, these antibodies may provide an important tool to understand the structural and functional aspects of fimbrin molecules.

Acknowledgments

The authors are thankful to Phillip Scheinberg and Dr. Monika V. de Arruda for their help, Ms. Joanne Davis for her secretarial expertise, and Ms. Holly Ann Fossel and Ms. Elizabeth Wright for help with data entry and statistical analysis.

This research was supported in part by National Institutes of Health grants AM-11414, AM-05577, AR-36308, and CA-44704, the Lupus Foundation of America, and the Lucille P. Markey Foundation grants. Epaminondas C. De Mendonca was supported by grants from CNPq, Brazil.

References

1. Alberto, B., D. Pray, J. Lewis, M. Raff, K. Roberts, and J. D. Watson, editors. 1983. The cytoskeleton. *In* Molecular Biology of the Cell. Garland Publishing, New York. 549–610.

2. Wang, E. 1985. Intermediate filament associated proteins. *Ann. NY Acad. Sci.* 455:32–56.

3. Goldmann, R. D., A. Milsted, J. A. Schloss, J. Starger, and M. J. Yerna. 1979. Cytoplasmic fibers in mammalian cells: cytoskeletal and contractile elements. *Annu. Rev. Physiol.* 41:703–722.

4. Weber, K., and M. Osborn. 1985. The molecules of the cell matrix. *Sci. Am.* 253:110–120.

5. Pollard, T. D., and J. A. Cooper. 1986. Actin and actin-binding proteins. A critical evaluation of mechanisms and functions. *Annu. Rev. Biochem.* 55:987–1035.

6. de Arruda, M. V., S. Watson, C. S. Lin, J. Leavitt, and P. Matsudira. 1990. Fimbrin is a homologue of the cytoplasmic phosphoprotein plastin and has domains homologous with calmodulin and actin gelatin proteins. *J. Cell Biol.* 111:1069–1079.

7. Goldstein, D., J. Djeu, G. Latter, S. Burbeck, and J. Leavitt. 1985. Abundant synthesis of the transformation-induced protein of neoplastic human fibroblasts, plastin, in normal lymphocytes. *Cancer Res.* 45:5643–5647.

8. Bretscher, A., and K. Weber. 1980. Fimbrin, a new microfilament-associated protein present in microvilli and other cell surface structures. *J. Cell Biol.* 86:335–340.

9. Alcover, A., F. Ramirez-Lafita, C. Hernandes, A. Nieto, and J. Avila. 1985. Antibodies to vimentin intermediate filaments in sera from patients with SLE and RA: quantification by solid phase radioimmunoassay. *J. Rheumatol.* 12:233–236.

10. Blaschek, M. A., M. Boehme, J. Jouquan, A. M. Simitzis, S. Fifas, P. Le Goff, and P. Youinol. 1988. Relation of antivimentin antibodies to anticardiolipin antibodies in systemic lupus erythematosus. *Ann. Rheum. Dis.* 47:708–716.

11. Senecal, J. L., and J. Rauch. 1988. Hybridoma lupus autoantibodies can bind major cytoskeletal filaments in the absence of DNA-binding activity. *Arthritis Rheum.* 31:864–75.

12. Kurki, P., T. Helve, D. Dahl, and I. Virtanen. 1986. Neurofilament antibodies in systemic lupus erythematosus. *J. Rheumatol.* 13:69–73.

13. Robbins, M. L., S. E. Kornguth, C. L. Bell, T. Kalinke, D. England, P. Turski, and F. M. Graziano. 1988. Antineurofilament antibody evaluation in neuropsychiatric systemic lupus erythematosus: combination with anticardiolipin antibody assay and magnetic resonance imaging. *Arthritis Rheum.* 31:623–631.

14. Tan, E. M., A. S. Cohen, J. F. Fried, A. T. Masi, D. J. McShane, N. F. Rothfield, J. G. Schaller, N. Talal, and R. J. Winchester. 1982. Revised criteria for classification of systemic lupus erythematous. *Arthritis Rheum.* 25:1271–1277.

15. Eaton, R. B., G. Schnneider, and P. H. Schur. 1983. Enzyme immunoassay for antibodies to native DNA. Specificity and quality of antibodies. *Arthritis Rheum.* 26:52–62.

16. Schur, P. H., D. DeAngelis, and M. J. Jackson. 1974. Immunological detection of nucleic acids and antibodies to nucleic acids and nuclear antigens by counterimmunoelectrophoresis. *Clin. Exp. Immunol.* 17:209–218.

17. Schur, P. H., D. Marcus-Bagley, Z. Awdeh, E. J. Yunis, and C. A. Alper. 1990. The effect of ethnicity on major histocompatibility complex, complement allotypes and extended haplotypes in patients with systemic lupus erythematosus. *Arthritis Rheum.* 33:985–992.

18. Fronek, Z., R. R. Valle, R. B. Eaton, and P. H. Schur. 1986. The relationship between Sm and RNP antigens. *Clin. Immunol. Immunopathol.* 41:91–107.

19. SAS Institute, Inc. 1988. SAS/STAT User's Guide. Release 6.03. SAS Institute, Inc., Cary, NC. 189–282.

20. Kurki, P., T. Helve, and I. Virtanen. 1983. Antibodies to cytoplasmic intermediate filaments in rheumatic diseases. *J. Rheumatol.* 10:558–562.

21. Osung, O. A., M. Chandra, and E. J. Holborow. 1982. Antibody to intermediate filaments of the cytoskeleton. *Ann. Rheum. Dis.* 41:69–73.

22. Senecal, J. L., S. Fortin, A. Roussin, and F. Joyal. 1981. Anticytoskeletal autoantibody to microfilament anchorage sites recognizes novel focal contact proteins. *J. Clin. Invest.* 80:778–785.

23. Youinou P., P. LeGoff, C. B. Colaco, J. Thivolet, D. Tater, J. Viac, and M. Shipley. 1985. Antikeratin antibodies in serum and synovial fluid show specificity for rheumatoid arthritis in a study of connective tissue diseases. *Ann. Rheum. Dis.* 44:450–454.

24. Osung, D. A., M. Chandra, and E. J. Holborow. 1982. Intermediate filaments in synovial lining cells in rheumatoid arthritis and other arthritides are of vimentin type. *Ann. Rheum. Dis.* 41:74–77.

25. Bohme, M. J., D. A. Evans, M. A. Miles, and E. J. Holborow. 1986. Occurrence of autoantibodies to intermediate filament proteins in human visceral leishmaniasis and their induction by experimental polyclonal B-cell activation. *Immunology.* 59:583–588.

26. Whitehouse, J. M., N. Ferguson, and G. A. Currie. 1974. Autoantibodies to micro-tubules in infectious mononucleons. *Clin. Exp. Immunol.* 17:227–235.

27. Pedersen, J. S., B. H. Toh, S. A. Locarnini, I. D. Gust, and G. N. Shyamala. 1981. Autoantibody to intermediate filaments in viral hepatitis. *Clin. Immunol. Immunopathol.* 21:154–161.

28. Lidman, K., A. Biberfeld, G. A. Fagraeus, R. Norberg, R. Torstensson, G. Utter, L. Carlsson, J. Luca, and U. Lindberg. 1976. Anti-actin specificity of human smooth muscle antibodies in chronic active hepatitis. *Clin. Exp. Immunol.* 24:266–272.

29. McCarty, G. A., D. W. Valencia, M. J. Fritzler, and F. A. Barada. 1981. A unique antinuclear antibody staining only the mitotic spindle apparatus. *N. Engl. J. Med.* 305:703.

30. Sotelo, J., C. J. Gibbs, and D. C. Gajdusek. 1980. Autoantibodies against exonal neurofilaments in patients with Kuru and Creutzfeldt-Jakob disease. *Science (Wash. DC).* 210:190–193.

31. Kataaha, P. K., S. M. Mortzavi-Milani, G. Russell, and E. J. Holborow. 1985. Anti-intermediate filament antibodies, antikeratin antibody, and antiperinuclear factor in rheumatoid arthritis and infectious mononuclesis. *Ann. Rheum. Dis.* 44:446–449.

32. Alcover, A., J. Molano, J. Renart, A. Gil-Aguaedo, A. Nieto, and J. Avila. 1984. Antibodies to vimentin intermediate filaments in sera from patients with systemic lupus erythematosus. *Arthritis Rheum.* 27:922–928.

33. Itoh, Y., M. D. Radar, and M. Reichlin. 1990. Heterogenetiy of the RO/SSA antigen and autoanti-Ro/SS-A response: evidence for 4 antigenically distinct forms. *Clin. Exp. Immunol.* 81:45–51.

34. Provost, T. T. 1979. Subsets in systemic lupus erythematosus. *J. Invest. Dermatol.* 72:110–113.

35. Andre-Schwartz, J., S. K. Datta, Y. Shoenfeld, D. A. Isenberg, B. D. Stollar, and R. S. Schwartz. 1984. Binding of cytoskeletal proteins by monoclonal anti-DNA lupus autoantibodies. *Clin. Immunol. Immunopathol.* 31:261–271.

36. Griffing, W. L., S. Moore, H. S. Luthra, C. H. Mckenna, and C. G. Fathman. 1980. Associations of antibodies to native DNA with HLA-DRw3. A possible major histocompatibility-linked human immune response gene. *J. Exp. Med.* 152:319–325.

37. Schur, P. H., I. Meyer, M. Garovoy, and C. B. Carpenter. 1982. Associations between systemic lupus erythematosus and the major histocompatibility complex: clinical and immunological considerations. *Clin. Immunol. Immunopathol.* 24:263–275.

38. Howard, P. F., M. C. Hochberg, W. B. Bias, F. C. Arnett, and R. H. McLean. 1986. Relationship between C4 null genes, HLA-D region antigens, and genetic susceptibility to SLE in Caucasian and Black Americans. *Am. J. Med.* 81:187–193.

39. Bell, D. A., and R. J. Maddison. 1980. Serologic subsets in systemic lupus erythematosus: an examination of autoantibodies in relationship to clinical features of disease and HLA antigens. *Arthritis Rheum.* 23:1268–1273.

40. Hochberg, M. C., R. E. Boyd, J. M. Ahearn, F. C. Arnett, W. B. Bias, T. T. Provost, and M. B. Stevens. 1985. Systemic lupus erythematosus: a review of clinico-laboratory features and immunogenetic markers in 150 patients with emphasis on demographic subsets. *Medicine (Baltimore).* 64:285–295.

41. Reichlin, M. 1981. Current perspectives on serological reactions in SLE patients. *Clin. Exp. Immunol.* 44:1–10.