Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 2956–2959, May 1987
Immunology

Presence of antibodies against a cell-surface protein, cross-reactive with DNA, in systemic lupus erythematosus: A marker of the disease

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Contributed by François Jacob, January 21, 1987

ABSTRACT **Antibodies against a cell-surface protein, cross-reactive with double-stranded DNA, were detected in the serum of 25 patients with active human systemic lupus erythematosus (SLE), defined on the basis of the revised American Rheumatism Association classification. Among these sera, two did not display anti-DNA antibodies, as shown by Farr assay, solid-phase radioimmunoassay, and *Crithidia luciliae* test. Five other SLE patients were consecutively studied in active and remission states. Antibodies against the protein were detected in the serum of the 5 SLE patients when they were in active phase but not in the serum of the same patients in inactive phase of the disease. The anti-protein antibodies were not found in the serum of 10 inactive SLE patients or in the sera of 10 normal human controls, 10 patients with rheumatoid arthritis, 5 patients with scleroderma, and 4 patients with primary sicca syndrome. Taken together, these results strongly suggest that antibodies against this cell-surface protein could provide a better diagnosis marker and activity index than anti-DNA antibodies in SLE.**

Anti-double-stranded DNA (dsDNA) antibodies are the hallmark of systemic lupus erythematosus (SLE). They are present in the majority of cases of SLE and usually are not found in other diseases. They are assumed to play a major role in the pathogenesis of the disease (1).

However, three observations remain unexplained. (*i*) There is only a relative correlation between anti-dsDNA antibody titer and disease activity. (*ii*) DNA has never been proven to be detected within circulating immune complexes or renal eluates of lupus mice and humans when critical methods of DNA chemical dosage are used. (*iii*) It has been proven paradoxically difficult to induce the production of anti-dsDNA antibodies in normal animals by *de novo* immunization with DNA (2).

Further light on the significance of anti-dsDNA that might explain these difficulties has arisen from the study of the cross-reactivities of monoclonal anti-single-stranded DNA (ssDNA) antibodies. It was shown by Schwartz's group that anti-ssDNA reacts with phospholipid, notably cardiolipin used in the syphilis diagnostic test (3).

Using monoclonal antibodies (mAbs) with strict specificity for dsDNA (not reacting with ssDNA), we have reported that monoclonal anti-DNA antibodies recognize a protein expressed at the surface of several cell types, notably T and B cells, erythrocytes, platelets, glomerular cells, and neuronal cells—all cells involved in SLE pathogenesis (4, 5). Five polypeptides have been characterized by immunoblot analysis with molecular masses ranging from 34 kDa to 14 kDa. These polypeptides probably derive from a unique cell-surface protein (6). These results have recently been confirmed (7). We have reported elsewhere the presence of (polyclonal) antibodies against this protein in MRL/lpr lupus mice sera (6). We present here evidence that such antibodies are present in human SLE. Because of the presence of these antibodies in sera of lupus mice and humans, we have called the protein lupus-associated membrane protein (LAMP) (8).

MATERIALS AND METHODS

Patients. Twenty-five patients with *active* SLE were studied. SLE was defined on the basis of the criteria of the American Rheumatism Association (9).

Clinically, the patients had arthralgia or arthritis in 92% of the cases, malar rash in 12%, discoid lupus in 12%, photosensitivity in 16%, and mouth ulcers in 24%. The severity of the disease was assessed by the presence of renal involvement in 68% of the cases, central nervous system involvement in 28%, and pericarditis and/or pleuritis in 24%. Leukopenia was present in 64% of the cases, lymphocytopenia in 40%, and thrombocytopenia in 8%.

All patients had antinuclear antibodies as defined by immunofluorescence tests performed on rat liver sections. All but two had anti-dsDNA antibodies as assessed by the Farr assay [performed as described (10)] (% binding activity ranging from 43% to 96% for sera diluted 1:10). The negativity of the two patients' sera in the Farr assay was confirmed by using solid-phase radioimmunoassay and by using *Crithidia luciliae* as an immunofluorescence assay as described (11). Anti-Sm antibodies (evaluated by immunodiffusion) were found in 4 of the 15 patients searched.

Five other SLE patients were consecutively studied in active and remission states, as summarized in Table 1, and 10 other SLE patients were studied in remission state. Control subjects included 10 patients with rheumatoid arthritis, 5 patients with scleroderma, 4 patients with primary sicca syndrome, and 10 normal subjects.

Detection of Anti-LAMP Antibodies. The technique is based on the immunoreplica analysis of patient's serum reactivity by immunoblot of an elastase supernatant of the IW 32 cell line known to contain significant amounts of LAMP (8). IW 32 is a mouse erythroleukemia cell line, which was kindly provided by N. Casadevall and O. Muller (12) (Hôpital Cochin, Paris). The elastase treatment consists in adding (for 7 min in ice) elastase (final concentration, 10 μg/ml) to 10^6 viable IW 32 cells in 1 ml of a 50 mM Tris·HCl buffer (pH 8.0) with 0.15 M NaCl. After the treatment with elastase, proteins

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Abbreviations: SLE, systemic lupus erythematosus; mAb, monoclonal antibody; dsDNA, double-stranded DNA; ssDNA, single-stranded DNA; LAMP, lupus-associated membrane protein.

Table 1. SLE patients in active and inactive phases

Clinical and biological data	Patient 1		Patient 2		Patient 3		Patient 4		Patient 5	
	A	R	A	R	A	R	A	R	A	R
General manifestation*	++	None	++	None	++	None	++	None	None	None
Major organ involvement†	DPGN	Histology ND	None	None	DPGN	None	None	None	DPGN, end-stage renal failure	Same
Minor organ involvement	Malar rash	None	Malar rash, mouth ulcers, arthritis	None	Malar rash	None	Arthritis	None	Arthritis	None
WBC	↓	N	↓	N	N	N	N	N	N	N
Proteinuria	+++	0	0	0	++	0	0	0	0	0
Complement	↓↓	↓	N	N	N	N	↓↓↓	N	N	N
N-DNA, %	96	15	83	24	66	11	58	14	95	21
Anti-LAMP	++	−	++	−	+++	−	+	−	++	−

A, active phase; R, remission; ND, not done; WBC, leukocyte count; N, normal. +, Mild involvement; ++, moderate; +++, severe. ↓, mild decrease; ↓↓, moderate; ↓↓↓, severe.
*Fever, weight loss, anorexia, asthenia.
†Central nervous system, diffuse proliferative glomerulonephritis (DPGN), vasculitis, hemolytic anemia.

released in the supernatants were collected after low-speed centrifugation (200 × *g* for 7 min).

Immunoreplica analysis was performed as described in detail (13). In brief, an aliquot of the elastase supernatant was subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis according to Laemmli. The proteins were then electrotransferred to a nitrocellulose sheet. Patients' sera were then incubated (1:20 dilution of the heat-inactivated sera). After 90 min of incubation at room temperature and washing, the binding of patients' antibodies to the antigens present in the immunoblot was revealed by addition of a peroxidase-labeled anti-human immunoglobulin (IgG) antiserum (14, 15). In the case of positive reaction, several bands corresponding to distinct polypeptides are visible. These polypeptides are then compared with those recognized by a monoclonal anti-dsDNA antibody previously described (4). This molecular mass was evaluated by reference to several markers (4). The intensity of the reaction with the patients' antibodies is evaluated in a semiquantitative fashion as +/++/+++.

λ phage DNA used for inhibition of the anti-LAMP reaction were obtained from Boehringer Mannheim GmBH.

RESULTS

Presence of Anti-LAMP Antibodies in Patients' Sera. The presence of antibodies against a cell-surface protein, cross-reacting with DNA in human SLE serum, was investigated. We have reported elsewhere that five major bands of 34 kDa, 33 kDa, 17 kDa, 16 kDa, and 14 kDa were detected in the elastase supernatant with PME77 mAb in immunoblot (Fig. 1, lane b). As shown in Fig. 1 (lanes c–j), these bands were detected in 25 human SLE sera diluted 1:20 using the elastase supernatant as antigen.

The reaction was variable with regard to the intensity and the number of bands revealed. All five bands were not always observed. This may be due to an extreme sensitivity of the protein to proteases depending on the experimental conditions. The reaction was strong (++, +++) for most SLE

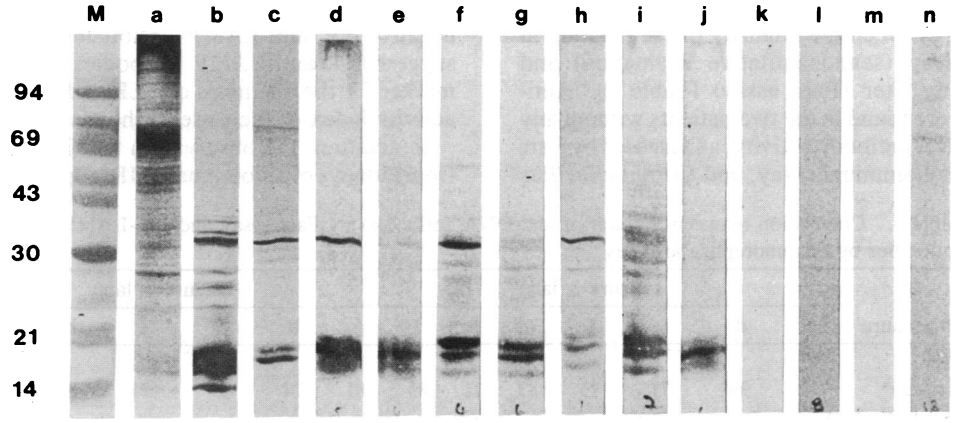

FIG. 1. Presence of anti-LAMP antibodies in eight SLE sera, using immunoreplica analysis: electrophoretic protein pattern and specific immunoreplica analysis of the elastase supernatant. M, marker proteins (molecular masses given in kDa); lanes: a, elastase supernatant, total protein pattern; b, elastase supernatant transferred to a nitrocellulose sheet and incubated with PME77 mAb; c–j, elastase supernatant transferred to a nitrocellulose sheet and incubated with eight different SLE sera; k–n, elastase supernatant transferred to a nitrocellulose sheet and incubated with normal human serum, rheumatoid arthritis serum, scleroderma serum, primary sicca syndrome serum, respectively, used as control. Note that the SLE serum, in lane h, was negative for anti-DNA antibodies by Farr assay, solid-phase radioimmunoassay, and *Crithidia luciliae* test.

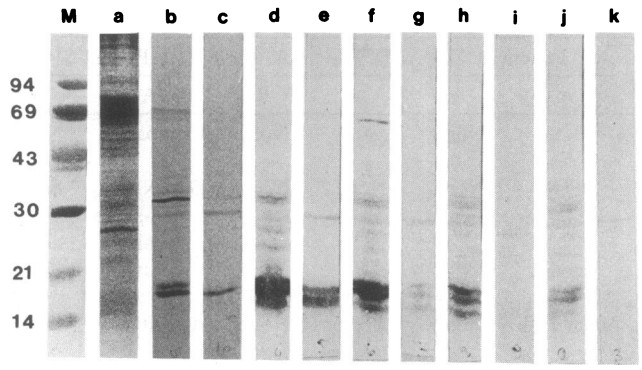

FIG. 2. Inhibition of the reaction of anti-LAMP antibodies with LAMP by dsDNA in five other human SLE sera, using immunoreplica analysis: electrophoretic protein pattern and specific immunoreplica analysis of the elastase supernatant. Lanes: M, marker proteins (molecular masses given in kDa); a, elastase supernatant, total protein pattern; b, d, f, h, and j, elastase supernatant transferred to a nitrocellulose sheet and incubated with SLE sera 1–5, respectively, diluted 1:20; c, e, g, i, and k, elastase supernatant transferred to a nitrocellulose sheet and incubated with SLE sera 1–5, respectively, diluted 1:20, preincubated with dsDNA. Note that, in the SLE serum 4, conventional anti-dsDNA antibodies assays were negative. However, the immunoreaction on nitrocellulose was inhibited by dsDNA, suggesting a superior sensitivity of the immunoreplica analysis to other assays usually performed to detect anti-DNA antibodies. The partial inhibition for sera 1, 2, and 3 could be due to the presence of antibodies against epitopes present on the LAMP molecule that do not cross-react with dsDNA.

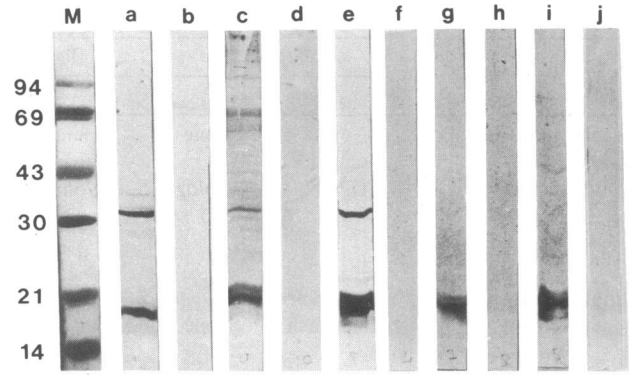

FIG. 3. Detection of anti-LAMP antibodies in the sera of five SLE patients in active and inactive states, using immunoreplica analysis: electrophoretic protein pattern and specific immunoreplica analysis of the elastase supernatant. Lane M, marker proteins (molecular masses given in kDa). Elastase supernatant was transferred to a nitrocellulose sheet and incubated with active SLE serum 1 (lane a), inactive SLE serum 1 (lane b), active SLE serum 2 (lane c), inactive SLE serum 2 (lane d), active SLE serum 3 (lane e), inactive SLE serum 3 (lane f), active SLE serum 4 (lane g), inactive SLE serum 4 (lane h), active SLE serum 5 (lane i), and inactive SLE serum 5 (lane j).

sera. The 17-kDa polypeptide gave the strongest reaction (as in the case of the PME77 mAb).

The anti-LAMP antibody reaction was partially inhibited after addition of 1 mg of DNA per ml (λ phage DNA) (Fig. 2). This partial inhibition could be explained by the presence of antibodies against epitopes present on LAMP molecule that do not cross-react with dsDNA.

Evolution of Anti-LAMP Antibodies in the Serum of SLE Patients in Active and Inactive Phases. As shown in Fig. 3, anti-LAMP antibodies were also detected in the serum of 5 other SLE patients who were in active phase but were not detected in the serum of the same patients in inactive phase of disease or in the serum of 10 inactive SLE patients. No reaction was observed with any of the control sera (10 normal subjects, 10 patients with rheumatoid arthritis, 5 patients with scleroderma, 4 patients with primary sicca syndrome).

Correlation with Anti-dsDNA Antibodies. No clear correlation was observed between the intensity of the reaction in the immunoblot assay (semiquantitative evaluation) and anti-dsDNA antibody titer (Farr assay) (Table 2). Anti-LAMP antibodies were found in the two patients without any detectable anti-dsDNA antibody activity, as assessed by Farr assay, solid-phase radioimmunoassay, and *Crithidia luciliae* assay. It is worth noting, however, that in spite of the absence of detectable dsDNA binding capacity, the reaction of the serum of these two patients with the polypeptides was decreased by incubation with DNA.

DISCUSSION

The presence of a high titer of antibodies directed against dsDNA is highly specific for SLE. There is, however, only a relative correlation between anti-DNA antibody titer and the clinical activity of the disease as defined by the clinical criteria of the American Rheumatism Association. In addition, there are SLE patients without anti-DNA antibodies.

We describe here a marker that might be more closely associated with SLE than anti-DNA antibodies. This is based on the detection by immunoreplica analysis of antibodies directed against LAMP, which previously has been shown to cross-react with dsDNA. In this paper, we demonstrate the presence of anti-LAMP antibodies in all sera from active SLE patients tested. It should be stressed that two of these sera did not display anti-DNA antibodies as shown by a series of standard assays. Conversely, all inactive SLE patients were negative for anti-LAMP antibodies. These data strongly suggest that anti-LAMP antibodies could provide a better marker for the diagnosis of SLE and could represent a good activity index of the state of the disease.

In addition, the observation that immunoglobulins eluted from kidneys of autoimmune MRL/lpr mice react specifically

Table 2. Correlation between the titer of anti-DNA antibodies by Farr assay and anti-LAMP antibodies by immunoreplica analysis

	Figure 1, lane								Figure 2, lane				
Procedure	c	d	e	f	g	h	i	j	b	d	f	h	j
Farr assay*	74	90	71	43	83	11	85	81	74	85	90	12	82
Immunoreplica analysis†	++	+++	+++	++	++	+	++	++	++	+++	+++	++	+

*The Farr assay, expressed in DNA binding (%), is considered to be positive in our test above 20% (control group + 3 SD).

†Immunoreplica analysis is only semiquantitative: +++, high reaction; ++, medium reaction; +, low reaction.

with this protein suggests the potential pathogenic role of the protein in the onset of lupus nephritis (6). Moreover, antibodies against this protein are also present, in large amount, in MRL/lpr and B/W mice sera (6). Most remarkably, we showed that LAMP is altered at the surface of spleen cells from MRL/lpr and B/W lupus mice in contrast to spleen cells from BALB/c and CBA/ca normal mice. These results suggest that this protein is modified by a change in its primary structure or by means of its association with other cell-surface proteins (6). Furthermore, we recently produced a polyclonal antiserum against LAMP by immunizing a rabbit with the protein from normal cells (8). We suggest that LAMP may act as a potent immunogen, instead of DNA itself, when the physiopathological conditions associated with the development of SLE are observed.

In contrast to the current hypothesis, we suggest, therefore, that LAMP may trigger, instead of DNA itself, an autoimmune response similar to the acetylcholine receptor in myasthenia gravis (16, 17). The binding of these antibodies to DNA might therefore be explained by a cross-reacting epitope(s) between DNA and this cell-surface protein. How could we interpret such a cross-reaction at the molecular level? More detailed information on the LAMP structure is necessary to elucidate this mysterious phenomenon.

We thank Dr. Marcel Francis Kahn and Dr. Bernard Weil for the generous gift of sera, Dr. Daniel Sereni for his suggestions, and Mrs. Jacqueline Primo for her excellent assistance. This work was supported by Institut National de la Santé et de la Recherche Médicale U 25 Grant 811050.

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