Binding of a Monoclonal Anti-DNA Autoantibody to Identical Protein(s) Present at the Surface of Several Human Cell Types Involved in Lupus Pathogenesis

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Abstract

A monoclonal anti-DNA antibody PME77, spontaneously produced in autoimmune B/W mice, has been found to recognize identical protein(s) present at the surface of several human cell types involved in the pathogenesis of systemic lupus erythematosus: glomeruli, platelets, erythrocytes, T and B cells, and neuronal tissue. Data indicate that protein(s) could represent a major stimulus or the target of anti-DNA autoimmunity and could account for tissue lesions observed in this disease.

Introduction

Most tissue lesions observed in murine and human systemic lupus erythematosus (SLE)[1] are considered to be related to the presence of anti-double-stranded deoxyribonucleic acid (dsDNA) antibodies that are spontaneously produced in large amounts. These antibodies are believed to be deposited in the form of DNA-anti-DNA complexes in many organs (1). However, it is difficult to produce antibodies to dsDNA by deliberate immunization (2). We recently reported that a murine monoclonal anti-DNA antibody (mAb) PME77 recognizes polypeptides present at the surface of the human B lymphoblastoid cell line Raji (3). Three of them are abondant and easily removed from the cell surface. We show here that this mAb reacts with five well-defined polypeptides at the surface of several cell types involved in the pathogenesis of SLE: glomeruli, platelets, erythrocytes, T cells (studied in the form of a T lymphoblastoid cell line CEM), and neuronal tissue. The antigenic determinant recognized by the PME77 mAb is thought to be present at the cell surface as evidenced by its disappearance after proteinase K treatment.

Methods

Mammalian cell types. The human lymphoblastoid B cell line (Raji) and the human lymphoblastoid T cell line (CEM) were obtained and maintained as previously described (4). Human erythrocytes were separated from mononuclear cells by centrifugation on Ficoll-Paque (Pharmacia, Inc., Piscataway, NJ) as described (5). Human platelets were provided by the blood bank of Necker Hospital, Paris, France.

Human glomeruli were isolated by Dr. L. H. Noel from Necker Hospital, according to a procedure based on the method described by Westberg and Michael (6). Normal kidney was perfused with Collins solution. The medulla was dissected away from the cortex and discarded. The cortex was then transferred to a 160-mesh stainless steel sieve. The tissue was forced through the sieve with moderate pressure using the bottom of a small flask and repeated washing with cold 0.85% sodium chloride solution, 0.5–1 liter for one adult size kidney. The pressing was continued for about 10 min per application or for 1 h per kidney. Material remaining on the screen was discarded. The sieved suspension was then poured through a 60-mesh sieve (pore size, 230 μm) to retain large tissue fragments, and finally through a 200 or 250-mesh sieve (opening, 74 or 61 μm). The material retained on the fine sieve was then extensively washed with cold 0.85% sodium chloride, 1–2 liter per kidney, until nothing but glomeruli, and possibly some tubular fragments attached to glomeruli, could be seen by phase contrast microscopy. This material was washed into a beaker, transferred to 50-ml plastic centrifuge tubes, and centrifuged at 1,080 *g* for 10 min. Finally, the sediment was washed four times in distilled water with centrifugation at 1,080 *g* at 4°C for 10 min. During the entire procedure, the tissue was carefully kept on ice.

Other tissues were isolated from rat including liver, pancreas, large intestine, testicle, and ovary. Membrane of rat neuronal tissue was kindly provided by Mrs. Ariane Monron from Pasteur Institute, Paris, France, and mouse teratocarcinoma line F9 was provided by Dr. F. Jacob from Pasteur Institute (7).

Murine monoclonal antibodies (mAb). The hybridoma PME77 secreting anti-DNA antibody was obtained after fusion between a nonsecreting myeloma line (P3 × 63 Ag 8.653) and (New Zealand Black × New Zealand White) F1 spleen cells. PME77 mAb was shown to be sharply specific for dsDNA (8).

Gel electrophoresis and immunoreplica analysis. Gel extracts were prepared following the procedure described by Garrels and Gibson (9). Cells were lysed at 4°C in a buffer containing 2 mM $CaCl_2$, 20 mM Tris-HCl, and 50 μg/ml staphylococcal nuclease (Boehringer Mannheim Biochemicals, Indianapolis, IN) adjusted at pH 8.8 at a cellular protein concentration of 1–2 mg/ml. Then 0.1 vol of 3% sodium dodecyl sulfate (SDS) 10% 2-mercaptoethanol was added at 4°C. The extract was treated with deoxyribonuclease and ribonuclease at 4°C (1 mg/ml of deoxyribonuclease I [Boehringer Mannheim Biochemicals] and 0.5 mg/ml of ribonuclease A [Boehringer Mannheim Biochemicals] in 50 mM $MgCl_2$, 0.5 M Tris-HCl, pH 7.0). All steps were performed in the presence of 0.1 mM phenylmethyl sulfonidefluoride and the mixture of proteases inhibitors described by Craig and Lancashire (10). Sodium dodecyl sulphate-polyacrylamide gel slab gels were run according to Laemmli (11). After electrophoresis, the immunoreplica technique was performed as described by Burnette (12), modified by Coudrier et al. (13). After electrotransfer of polypeptides, the nitrocellulose sheet was

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1. *Abbreviations used in this paper:* dsDNA, double-stranded deoxyribonucleic acid; mAb, monoclonal antibody; SLE, systemic lupus erythematosus.

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incubated with the supernatant of PME77 mAb secreting hydridoma for 90 min at room temperature. The antigen-PME77 mAb complexes were detected with the peroxidase technique. Molecular weight markers were phosphorylase b (93,000), bovine serum albumin (66,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,000), and lactalbumin (14,000).

Results

PME77 anti-DNA mAb binds to identical polypeptides present at the surface of different cell types. We have previously reported that PME77 mAb with strict specificity for dsDNA recognizes polypeptides present at the surface of Raji cells (3). To determine whether the same polypeptides were recognized on cell types involved in the pathogenesis of SLE, immunoreplica analysis was used. Electrophoresis of polypeptides extracted from different cell types was performed. Polypeptides thus separated were transferred to the nitrocellulose sheet. The supernatant of PME77 mAb was then added and incubated. As shown in Fig. 1, five bands were detected at 14,000, 16,000, 17,000, 33,000, and 34,000 in glomeruli extract. These bands were strongly reduced when PME77 mAb was preincubated with 1 mg/ml DNA, and not seen when using a DNA-unrelated mAb raised against a minor membrane protein of the golgi apparatus (14). To determine the cell surface accessibility of the antigenic determinant recognized by the PME77 mAb and to confirm its proteinic nature, a mild proteinase K treatment was applied to living cells as previously described in detail (3).

A proteinase K treatment rendered the cell unreactive to the PME77 mAb.

The same strategy was used to study the antigenic determinant recognized by the PME77 mAb on various cell types: platelets, erythrocytes, a T lymphoblastoid cell line CEM, neuronal tissue membrane, ovary, testicle, and teratocarcinoma line F9. The same five bands were detected in total cell extract, all sensitive to the proteinase K treatment (Table I).

A strong reaction was observed with glomeruli, T lymphoblastoid cell line, and teratocarcinoma. A weaker reaction was found with erythrocytes (Fig. 1), platelets, neuronal tissue membrane, ovary, and testicle. No reaction was observed with other tissue such as liver (Fig. 1), large intestine, and pancreas (Table I). These results suggest that the five polypeptides are expressed at different levels in the various cell types involved in lupus pathogenesis.

Discussion

Anti-DNA antibodies are the predominant autoantibodies in murine and human SLE. If one considers, however, that such antibodies are exclusively directed against DNA, it seems difficult to explain the paradox between the frequency with which these antibodies are spontaneously produced in large amounts, and the difficulties met to elicit their production by deliberate immunization by nucleic acid preparation in non-autoimmune animals.

The recognition by anti-DNA mAb of phospholipids gave

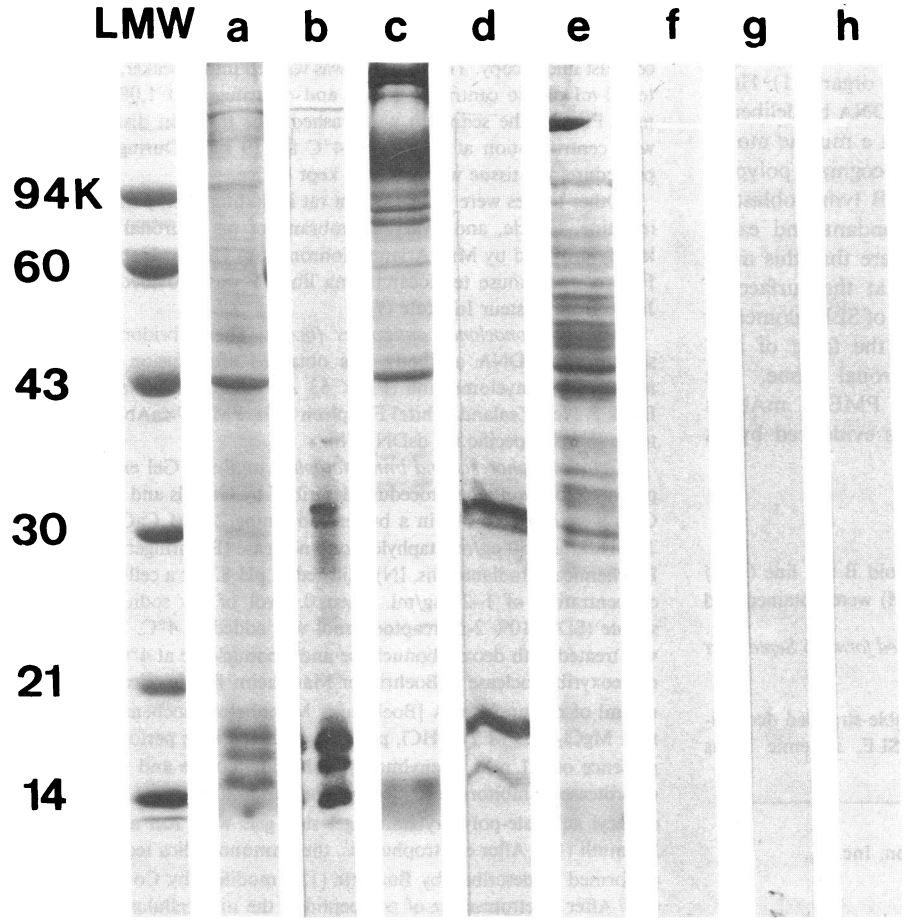

Figure 1. Electrophoretic protein pattern and specific immunoreplica analysis of human glomeruli extract, human erythrocyte extract, and rat liver extract. In each case, the gels were loaded with 20 μg of glomeruli extract, 50 μg of erythrocyte extract, and 50 μg of liver extract. (*a*) glomeruli extract, total protein pattern; (*b*) glomeruli extract transfer to a nitrocellulose sheet and incubated with PME77 mAb; (*c*) erythrocyte extract, total protein pattern; (*d*) erythrocyte extract transfer to a nitrocellulose sheet and incubated with PME77 mAb; (*e*) liver extract, total protein pattern; (*f*) liver extract transfer to a nitrocellulose sheet and incubated with PME77 mAb; (*g*) control with PME77 mAb preincubated with DNA; (*h*) control with anti-golgi mAb. The difference of the intensity of the reaction is only semi-quantitative, since cell extracts were analysed in separate experiments. However, these results indicate that different organs express variable amounts of polypeptides.

Table I. Binding of PME77 mAb to Five Polypeptides Present at the Surface of Different Cell Type Extracts Used at Different Concentrations

Cell type extracts	Strong reaction	Weak reaction	No reaction
Human platelets (50 μg)		+	
Human erythrocytes (50 μg)		+	
Human glomeruli (20 μg)	++		
Human T lymphoblastoid cell line CEM (20 μg)	++		
Human B lymphoblastoid cell line Raji (20 μg)	++		
Rat neuronal tissue membrane (50 μg)		+	
Rat ovary (50 μg)		+	
Rat testicle (50 μg)		+	
Rat liver (50 μg)			−
Rat large intestine (50 μg)			−
Rat pancreas (50 μg)			−
Mouse teratocarcinoma cell line F9 (20 μg)	++		

the first hint of a possible triggering mechanism (15). Our recent report that mAb with strict specificity for dsDNA recognizes polypeptides at the surface of Raji cells (3) provides another possible clue. Here, we demonstrate that this mAb recognizes identical polypeptides on the surface of different human cell types involved in SLE: glomeruli, erythrocytes, platelets, T cells (studied in the form of T lymphoblastoid cell lines CEM), and neuronal tissue membrane. The high affinity of the anti-DNA mAb for these polypeptides might account for some of the autoantibody cross-reaction observed in SLE, such as that found between anti-neuronal, anti-erythrocytes, and anti-lymphocyte antibodies (16), or that described between anti-lymphocyte and anti-nuclear antibodies (17). These results open the possibility that the triggering antigen in SLE could be a protein cross-reacting with DNA, rather than DNA itself, accounting for the tissue lesions observed in this disease. This hypothesis would account for the low immunogenicity of nucleic acids outlined above.

The strong reaction observed with teratocarcinoma cell line suggests that the polypeptides in question are present at a high concentration in undifferentiated cell types that could have important implications for the establishment of self-tolerance to the antigen(s) involved in human SLE in early stages of embryon immunologic development.

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