Lupus Anti-DNA Antibodies Bearing the 8.12 Idiotype Appear to Be Somatically Mutated

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Anti-DNA antibodies in systemic lupus erythematosus (SLE) sera were analyzed using an antiidiotype designated 8.12 which recognizes a determinant on lambda light chains highly expressed in SLE sera. Eight of ten normal individuals had peripheral blood lymphocytes which produced high-titered 8.12-positive antibodies, following transformation with Epstein Barr virus, implying that the 8.12-reactive sequence originates in the germline gene (GLG). Of 58 SLE sera, 32 contained elevated titers of 8.12-reactive antibodies. Twenty-three of these sera had 8.12-reactive anti-DNA antibodies, suggesting a strong correlation between 8.12 idiotype and DNA binding. Moreover, 20 of 26 8.12-reactive IgG antibodies and only 4 of 10 8.12-reactive IgM antibodies bound DNA (P < 0.05). These observations strengthen our previous findings in myeloma sera that DNA binding is associated with IgG isotype in the 8.12 idiotype system and suggest that the acquisition of anti-DNA reactivity in antibodies bearing the GLG idiotype 8.12 is achieved by somatic mutation, a feature of an antigen-driven response.

KEY WORDS: Anti-DNA antibodies; idiotype; somatic mutation; systemic lupus erythematosus.

INTRODUCTION

The mechanism of anti-DNA antibody production in systemic lupus erythematosus (SLE) patients is controversial. On the one hand, there is evidence that suggests that the anti-DNA specificity is encoded by germline variable-region genes and that the anti-DNA antibodies of SLE have features in common with antibodies induced by nonspecific polyclonal stimulation of B cells. This is indicated by the finding of anti-DNA antibodies with the germline gene (GLG) sequence (1, 2) and the finding that B-cell lines obtained from healthy individuals are capable of producing anti-DNA antibodies (3, 4). On the other hand, data from both murine and human studies show lupus anti-DNA antibodies to be somatically mutated, with sequences which differ significantly from the homologous germline genes (5–8). In these antibodies, the ratio of replacement to silent mutations suggests an antigen-selected response. Most of the studies on the origin of anti-DNA antibodies have been performed using monoclonal antibodies obtained from B-cell hybridomas, from Epstein Barr virus (EBV)-transformed lines, or from myeloma proteins. The results are thought to correspond to anti-DNA antibody generation in SLE. However, many of the human hybridoma antibodies studied were of the immunoglobulin M (IgM) isotype, polyspecific and with a low affinity for antigen, while "pathogenic" SLE anti-DNA antibodies are usually IgG, less broadly polyspecific, and have a high affinity for DNA. We therefore set out to study the origin of anti-DNA antibodies by directly analyzing 8.12-positive antibodies in SLE patients' sera. The 8.12 idiotype is present on lambda light chains of anti-DNA antibodies and is highly expressed in 50% of SLE sera (9).
MATERIALS AND METHODS

Production and Maintenance of EBV-Transformed B-Cell Lines

Blood from normal individuals (20 ml) was collected in heparinized syringes and left for 3 hr at room temperature. The white blood cells were separated from the red blood cells, diluted 1:2 in saline, layered on Ficoll-Paque (5 ml; Pharmacia, Sweden), and centrifuged (400g for 10 min). The lymphocytes were collected, washed, and counted. Ten million lymphocytes were added to 5 ml of medium containing cyclosporin A (0.5 μg/ml; Sandoz, Switzerland). Five milliliters of EBV-containing supernatant (from Marmoset cell line B95-8, donated by M. Steinitz, Israel) was added after 4 hr in order to transform the B lymphocytes. After 2-3 weeks of incubation (37°C, 5% CO2) the cells were fed for the first time and weekly thereafter with medium (RPMI 1640, Bio-Lab, Israel) supplemented with 10% fetal calf serum (FCS; Bio-Lab, Israel), penicillin (100 units/ml), streptomycin (100 μg/ml), Hepes buffer (20 mM), and glutamine (20 mM). Supernatant was collected 6 weeks after the transformation.

Generation and Purification of 8.12 Antidiotype

The generation and characterization of 8.12 antidiotype has been described previously (9, 10). Briefly, anti-double-stranded DNA antibodies were purified from a lupus patient and used to immunize BALB/c mice. Spleen cells of these mice were fused with cells of a nonproducing mouse myeloma cell line (P3x63/Ag 8.653). The 8.12 hybridoma antibody, an IgG1, κ, was shown to react with the immunizing antigen more than with normal immunoglobulin. The 8.12-reactive site was found to reside on lambda light chains and to be associated exclusively with cationic antibodies. Approximately one-third to one-half of 8.12-reactive antibodies in SLE serum are DNA binding (9).

Ascites fluid containing 1-10 mg/ml of 8.12 antidiotype was used to obtain purified 8.12. The immunoglobulin was precipitated using ammonium sulfate (50% final saturation). The mixture was centrifuged (10000 rpm for 10 min at 4°C) and the pellet obtained was washed 2× with ammonium sulfate (50% saturated), dissolved and dialyzed against Tris buffer (0.5 M NaCl, 0.05 M Tris, pH 7.8), and subjected to Sephacryl S-200 chromatography in Tris buffer. The class and purity of the pooled immunoglobulin were tested by electrophoresis and enzyme-linked immunosorbent assays (ELISA).

8.12 Reactivity of Sera

The 8.12 reactivity of sera was assayed using an ELISA. Sera diluted 1:1000 in saline were used to coat microplates. The wells were blocked with 5% FCS in saline, incubated with 8.12 ascites, diluted 1:300 in saline Tween 0.05%, and subsequently incubated with alkaline phosphatase-conjugated goat anti-mouse IgG (1:1000 in saline Tween 0.05%). In each step 100 μl/well of reagent was added and an incubation time of 30-60 min at 37°C was allowed. Plates were rinsed after each step with saline Tween 0.05%. Alkaline phosphatase substrate (disodium p-nitrophenyl phosphate, 5 mg/ml diluent) was added and the plates were read by ELISA reader at 405 nm. All the reagents for the ELISA assays were purchased from Sigma Chemical Co. (St. Louis, MO), unless otherwise specified. Controls were 8.12-reactive sera tested previously by radioimmunoassay and 10 normal sera. In all ELISA assays reactivity was considered positive if it exceeded the mean reactivity of the negative controls or of normal sera by 2 standard deviations.

8.12 Reactivity of Cell Line Supernatants

The 8.12 reactivity of supernatants from EBV-transformed cells was determined using ELISA. Purified 8.12 was used to coat microtiter plates (1 μg/well). The subsequent steps were block, addition of supernatants (with Tween 0.05%), addition of anti-human lambda-chain antibody conjugated to peroxidase (1:5000 in saline Tween 0.05%), and addition of substrate (O-phenylenediamine dihydrochloride, 0.6 mg/ml diluent). Incubation and washing of the plates were performed as above. For controls we used purified myeloma proteins (1 μg/well) that were obtained from myeloma sera using preparative isoelectric focusing gels as published elsewhere (11).

Determination of the Isotype Class of 8.12-Reactive Antibody

The 8.12-reactive sera (diluted 1:2000 in 0.05% saline Tween) or supernatants (with Tween 0.05%) were added to microtiter wells coated with the Journal of Clinical Immunology, Vol. 12, No. 1, 1992