Deoxyribonuclease-Inhibitory Antibodies in Systemic Lupus Erythematosus

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Abstract
Deoxyribonucleases (DNases) are key enzymes for digesting DNA. Abnormalities in the function of these enzymes may contribute to the development of anti-DNA antibodies in systemic lupus erythematosus (SLE). In this study, we used bovine DNase 1-coated ELISA plates to screen anti-DNase antibodies in SLE patients. About 62% of the sera of SLE patients (63/101) were positive for anti-DNase antibodies compared to only 8% of normal controls (8/98). A positive correlation was also found between the concentrations of anti-DNase and anti-DNA antibodies in sera of SLE patients. Affinity-purified anti-DNase immunoglobulin G (IgG) from pooled sera of SLE patients bound to bovine DNase as well as DNA. A synthetic peptide, corresponding to the catalytic site of DNase, was able to completely inhibit the binding of anti-DNase IgG to DNase. In addition to bovine DNase, the anti-DNase IgG also bound to and inhibited the enzymatic activities of DNase present in streptococcal supernatants and human urine. Immunization of lupus-prone NZB/NZW mice with bovine DNase enhanced the production of anti-DNase and DNA antibodies, and accelerated the occurrence of proteinuria. Taken together, these results suggest that DNase-inhibitory antibodies which recognize a conserved epitope near the catalytic site of DNase may act in the pathogenesis of SLE.

Introduction
Systemic lupus erythematosus (SLE) is a prototypical systemic autoimmune disease, which is characterized by the presence of antinuclear antibodies (ANAs) directed against DNA and nucleosomes. The resulting immune complexes are deposited in blood vessels and filter organs such as joints and the kidney, causing vasculitis, arthritis, and glomerulonephritis. The etiology of SLE is unknown, but several studies have suggested that inadequate clearance of potential autoantigens, such as nuclear DNA-protein complexes, after cell death may contribute to the loss of self-tolerance in SLE [21].

Deoxyribonuclease (DNase), which occurs in almost all living organisms, is a group of enzymes capable of hydrolyzing DNA, and at least two genes, DNase I and II, are known. Bovine pancreatic DNase I was the first discovered and is the best-characterized DNase [6, 11]. It consists of a single polypeptide of 260 amino acids and
contains two disulfide bridges. The crystal structure of bovine DNase I, complexed to a short oligonucleotide, has been resolved. An exposed loop of DNase I (amino acids 73–78, Arg-Asn-Ser-Tyr-Lys-Glu) binds to the minor groove of DNA, probably through electrostatic interactions [10, 16, 17]. In addition, the active site of DNase is phylogenetically highly conserved among DNase from different species. As in cows, human DNase also exhibits polymorphism and can be detected in different tissues such as sera, urine, the kidney, liver, and pancreas [4, 6]. Previous studies have shown that there are low serum and urine DNase activities in both SLE patients and SLE-prone NZB/NZW mice [2, 8]. Moreover, DNase I-deficient mice generated by gene targeting show the classical symptoms of SLE, which are the presence of ANAs and glomerulonephritis [9]. Mutation of DNase I gene has also been found in some SLE patients [23], although not all SLE patients show defects in DNase genes [1, 15, 19]. Therefore, multiple factors are probably involved in causing the decreases in serum DNase activity in SLE. In this study, we examined the prevalence of anti-DNase antibodies in SLE patients and used an affinity column to purify anti-DNase antibodies from sera of SLE patients to analyze their antigenic specificity and their effects on DNase enzymatic activity. Finally, NZB/NZW mice were immunized with DNase to study the pathogenic effects of anti-DNase antibodies in the disease development of SLE. Results from these studies indicate that anti-DNase antibodies in SLE may interfere with DNase activity and accelerate the disease process.

Materials and Methods

Human Sera

Serum samples were collected from 101 SLE patients fulfilling at least four of the revised 1982 criteria for SLE [18] and were stored at -70 °C. There were 85 females and 16 males, ranging in age from 4 to 64 years with a mean of 30 years. In addition, 98 normal serum samples without ANAs were used as controls.

Bovine DNase I

Bovine DNase I was purchased from Sigma Chemical Co. (St. Louis, Mo., USA). SDS-PAGE and zymography, respectively, confirmed the purity and activity of this enzyme. We confirmed that no DNA contamination existed in the bovine DNase I preparation because the bovine DNase I solution (1 mg/ml) showed no absorption at 260 nm, and because ethidium bromide staining of this DNase solution run on an agarose gel was also negative (data not shown).

Synthetic Peptides

Synthetic peptide (DNase peptide) corresponding to the catalytic domain of DNase (amino acids 73–78) [12] was synthesized by the Peptide Synthesis Center, National Science Council, Taipei, Taiwan. The sequence of this peptide is Ala-Arg-Asn-Ser-Tyr-Lys-Glu-Ala. An additional peptide (Glu-Leu-Lys-Cys-Tyr-Thr-Cys-Lys-Glu) was used as a negative control. The purity of these peptides was confirmed by HPLC and amino acid analysis.

Mice Immunization

Five- to 6-week-old female NZB/NZW mice were used in this study. These mice were originally purchased from Jackson Laboratory (Bar Harbor, Me., USA) and bred in the Laboratory Animal Center, National Cheng Kung University. DNase (50 μg/mouse) in complete Freund’s adjuvant was intraperitoneally injected into 6-month-old NZB/NZW mice. At 2 and 4 weeks, these mice were boosted with DNase I in incomplete Freund’s adjuvant by the same route as the primary immunization. Sera were collected from the tail of the mice at the indicated times. In addition, urine was collected from each group of mice at the indicated ages. The protein level in the urine was measured with the BCA protein assay (Pierce, Rockford, Ill., USA).

Purification of Immunoglobulin by Protein A and DNase Affinity Column

Immunoglobulin G (IgG) from normal sera was purified using a protein A affinity column (Pharmacia Biotech, Piscataway, N.J., USA). Unbound components were washed away with 0.85% saline. Bound IgG was then eluted with 0.1 M glycine (pH 3.0), and the pH of the eluent was neutralized to 7.0 with 1 M Tris (pH 8.0). For purification of anti-DNase antibodies, sera from SLE patients with high anti-DNase antibody titers were pooled and passed through a DNase affinity column, then through a protein A column. Briefly, bovine DNase I was conjugated to Sepharose 4B (Pharmacia Biotech) according to the manufacturer’s instructions. Pooled sera were passed through the DNase affinity column and eluted with 3 M MgCl₂. The eluted proteins were dialyzed against PBS, and anti-DNase IgG was further purified by the protein A column. The concentration of immunoglobulin was adjusted by ultrafiltration (Amicon, Beverly, Mass., USA) and determined by BCA protein assay.

Enzyme-Linked Immunosorbent Assay

Ninety-six-well flat-bottom enzyme-linked immunosorbent assay (ELISA) plates (Nunc, Denmark) were coated with 100 μl of either bovine DNase I (0.05 mg/ml in PBS) or synthetic peptides for 2 h at 37 °C. After washing with PBS, the plates were blocked with blocking buffer (1% bovine serum albumin in PBS) for 1 h at 37 °C. Then either 100 μl of diluted serum (100-fold in blocking buffer) or affinity-purified anti-DNase IgG was added and incubated for 2 h at 37 °C. Bound antibodies were detected by horseradish peroxidase-conjugated goat anti-human Ig or anti-mouse Ig antibodies (Sigma) followed by OPD substrate (Sigma). ELISA plates were read on a Vmax microplate reader (Molecular Device, Menlo Park, Calif., USA) at 490 nm. To detect DNA cross-reactive antibodies, plates were first precoated with 100 μl of methylated BSA (10 μg/ml in 1 N acetic acid and PBS) for 2 h at 37 °C and then coated with 100 μl of calf thymus deoxyribonucleic acid (Sigma; 2.6 μg/ml in PBS). In the competitive inhibition assays, anti-DNase IgG (0.5 mg/ml) was preincubated with different amounts of DNase peptide or control peptide for 1 h at 37 °C before being added to the DNase- or DNA-coated ELISA plates.