Cold-reacting Antinuclear Factor in Sera from Patients with Primary Glomerular Diseases

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Antinuclear factor (ANF) was determined in sera from patients with primary glomerular diseases to evaluate the autoimmune nature of this disorder. Sera from 61 patients with various types of glomerular disorders were assayed for ANF by the fluorescent antinuclear antibody (FANA) technique at 37°C and 4°C. Thirty healthy adults served as controls. Cold-reacting ANF, which was detected predominantly in the class of IgM, was observed in 54 out of 61 patients with various types of primary glomerular disorders. The nuclear staining pattern was "speckled". Sera from healthy adults did not show any positive signs of ANF. It is suggested that some autoimmune mechanisms may be involved in the development of primary glomerular diseases.

(Key Words: Glomerulonephritis, IgM, Antinuclear factor)

INTRODUCTION

Large numbers of renal biopsies have been studied by immunofluorescent antibody techniques in recent years. Although granular deposits of immunoglobulin and complement are observed in the glomeruli from patients with various primary glomerular diseases, responsible antigens have been identified only in a minority of such patients.

The purpose of this study was to determine antinuclear factors in sera from patients with primary glomerulonephritis in order to evaluate the autoimmune nature of this disorder.

MATERIALS AND METHODS

Individuals studied

Sixty one patients with primary glomerular diseases including 4 patients with minimal change nephrotic syndrome, 28 patients with IgA nephropathy, 9 patients with mesangial proliferative glomerulonephritis, 6 patients with membranoproliferative glomerulonephritis, 5 patients with membranous nephropathy and 9 patients with benign recurrent hematuria were examined. There were 31 male and 30 female patients, and their ages ranged from 16 to 48 years. Thirty healthy adults, ranging in age from 16 to 38, served as controls.

Diagnosis of these diseases was confirmed histologically by renal biopsies. Venous blood was allowed to clot at room temperature, and serum, obtained within 4 hours, was aliquoted and stored at -20°C.

Indirect fluorescent antibody test

The substrates for the test were mouse kidney, liver, and spleen and human kidney and leukocytes. Tissue sections from the cryostat and smears of leukocytes were air-dried, immersed in 90% ethanol for 10 min at room temperature, and air-dried again. Test sera were applied to the tissue sections or smears, and reaction was carried out in a moist chamber at 37°C for 30 min or at 4°C overnight. The sections and smears were washed three times for 10 min each in PBS (pH 7.2) and fluorescein-conjugated antiserum was applied to the sections at 4°C overnight. The sections and smears were washed again in PBS,
mounted in 90% glycerol in saline, and examined with a Zeiss fluorescence microscope (Model 9902; Carl Zeiss, Inc., New York, N.Y.).

Antibody preparations used for the fluorescent ANF studies were FITC-conjugated rabbit anti-sera to human IgG, IgA, and IgM, heavy chain-specific (Behringwerke AG, Marburg/Lahn, W. Germany). Monospecificity of the antisera was tested by immunoelectrophoresis and Ouchterlony double immunodiffusion. Only those with molar fluorescein/protein ratios between 1.5 and 2.9 were employed.

Interpretation

Titers: the presence of nuclear fluorescence in the greatest dilution was expressed as dilution titers.

Immunofluorescent nuclear patterns: staining patterns were recorded as homogenous, speckled, shaggy, and nucleolar.

Absorption studies

Extractable nuclear antigens were isolated from rabbit thymus powders (Pel-Freez Biological Inc., Rogers, Ark. U.S.A.) by the method of Tan and Peebles (7). Protein was determined by the method of Lowry and his associates (1). Soluble pellets containing approximately 2.5 mg of ENA were prepared and added to 0.5 ml of serum. This mixture was incubated at 4°C for 3 hr. Following centrifugation, the supernatant serum was removed and tested for ANF activity.

RESULTS

Temperature of incubation

None of the sera from normal controls or patients with primary glomerular diseases positive stained positively at 37°C. However, among 50 cases of healthy persons tested at 4°C, three stained weakly when mouse kidney or liver sections were used as a substrate. In these healthy subjects, titers did not exceed 1:10. Sera were considered positive if nuclear immunofluorescence of at least 1+ was present at a dilution of 1:10.

The distribution of the sero-positive patients for FANA is shown in Table 1. IgG-FANA was found in one, IgA-FANA was in two, and IgM-FANA in 54 patients sera when mouse kidney or liver sections were used as a substrate. The speckled nuclear staining pattern, as can be seen in Fig. 1, was detected in all positive sera from patients with primary glomerular diseases. This speckled nuclear staining pattern was not altered by dilution of the patients' sera. The factor in patients with these disorders could be completely removed from serum by absorption with ENA.

Substrate

Cold-reacting ANF in sera from patients with primary glomerular diseases was detected frequently when mouse kidney or liver sections, whereas no FANA were demonstrated with mouse spleen sections or human leukocyte smears.

DISCUSSION

Pathogenesis of primary glomerulonephritis remains obscure. It has been postulated that some antigen-antibody complexes are responsible for its development, although the responsible antigens have only been identified in a minority of patients. Recently, cold-reacting antibody to extractable nuclear antigens were found in sera from patients with IgA nephropathy (2). In this study, a cold-reacting ANF was detected in sera from 89% of patients with various types of primary glomerulonephritis using kidney or liver sections as substrates. Although cold-reacting ANF was observed in some healthy adults, the titer of ANF in such healthy adults was always less than 1:10. The FANA technique may be more sensitive than the FANA screening test at 37°C or room temperature and thus can be presumed to pick up antibodies with weak affinity. Furthermore, this procedure might detect additional immune precipitates distinct from the usual warm-reacting ANF. Of course, no autoantibodies in sera from patients with primary

Abbreviations:

antinuclear factor (ANF), antinuclear antibody (ANA), phosphate-buffered saline (PBS), extractable nuclear antigen (ENA), Fluorescein isothiocyanate (FITC), fluorescent antinuclear antibody (FANA), minimal change nephrotic syndrome (MCNS), proliferative glomerulonephritis (PGN), membranoproliferative glomerulonephritis (MPGN), membranous nephropathy (MN), benign recurrent hematuria (BRH), mixed connective tissue disease (MCTD).
glomerulonephritis have been detected by routine clinical tests. The patients with primary glomerulonephritis in this study showed no clinical feature of MCTD, as described by Sharp and associates (5, 6) and did not meet the ARA criteria for SLE. The activity of ANF in sera from patients with MCTD or SLE is predominantly in the class of IgG, whereas the activity of cold-reacting ANF is in the class of IgM. (Cold-reacting ANF was not detected when mouse spleen or human leukocytes were used as substrates. It is suggested that the cold-reacting ANF in sera from patients with primary glomerulonephritis does not react with nuclei obtained from lymphocytes or neutrophils. The absence of ANF specific to leukocytes is a mirror image of ANF observed in some patients with SLE. It is postulated that such absence of leukocyte-specific ANF might reflect different antigenic specificities among kidneys, liver, spleen, and leukocytes. It has already been emphasised that the development of chronic immune-complex nephritis requires a persistent exposure to antigen and the appropriate antibody response to it. In spontaneous chronic nephritides of laboratory animals, chronic viral infection has been described (3, 4). In human glomerulonephritis, it has been sporadically reported that these primary glomerulonephritides are mediated by viral antigen-antibody complexes, although hard evidence is not yet definitive. Although, there are no data concerning the in vivo activity of these cold-reacting ANF in sera from patients with glomerulonephritis, it is possible that they interfere with the normal balance of the immunological environment and thus favor development of autoimmune diseases. It is suggested that the exposure to certain antigens that have similar antigenecity to extractable nuclear antigens might be responsible in the development to primary glomerulonephritis.

ACKNOWLEDGEMENT

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REFERENCES

Table 1  FANA assay for anti-ENA antibodies in patients with various types of primary glomerular diseases.

<table>
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<th>Diagnosis</th>
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<th>Number positive</th>
<th>Percent positive</th>
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<tr>
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<tr>
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Fig. 1  The speckled nuclear staining demonstrated by serum from patients with primary glomerular diseases.