Proc EDTA–ERA (1985) Vol 22

IMMUNOCHEMICAL CHARACTERIZATION OF CATIONIC IgG IN PATIENTS WITH MEMBRANOUS NEPHROPATHY AND DECLINING RENAL FUNCTION

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Summary

Abnormal cationic IgG has been isolated from the serum of three patients with membranous nephropathy at the time renal function was declining in these patients. The IgG bands had isoelectric points of 8.6, 8.6 and 9.0 and all were of the IgG_1 subclass. An anti-idiotypic antiserum raised to the IgG (FS) band and adsorbed with normal human IgG was found to cross react with the other two isolated IgGs (MS and BB) but not with IgG_1 subclass paraproteins purified by a similar method. The cross reacting epitope may be a marker of disease activity in patients with membranous nephropathy.

Introduction

The uniformity of the histopathological appearance of granular deposits of IgG which decorate the subepithelial aspect of the glomerular capillary wall in membranous nephropathy is misleading. Experimental animal models have identified a variety of different mechanisms which produce the same histopathological pattern of IgG deposition [1–3].

Several models have demonstrated the crucial role of charge in the facilitated deposition of cationic macromolecules in the lamina rara externa. Cationized antigens were preplanted in the subepithelial site which, when later exposed to antibody produced by active immunization, allowed 'in situ' immune complex formation [2]. Interestingly, the reverse procedure of preplanting cationic antibody in the glomerular capillary wall and then introducing antigen also allowed in situ immune complex formation and produced the same characteristic histopathology [3].

The relevance of these pathogenic mechanisms to human membranous nephropathy requires investigation and we have been studying the charge spectrum of

serum IgG in patients with membranous nephropathy. In six of eight patients with membranous nephropathy and declining renal function, we have demonstrated by isoelectric focusing the presence of an abnormal cationic species of IgG [4]. We now report the isolation and partial characterization of this cationic IgG from three of these patients.

Methods

Five hundred millilitres of serum from patient FS, 150ml from patient MS and 130ml from patient BB were available for study. For each patient the serum was dialysed against 10mM sodium phosphate buffer pH 7.5 and applied to a column of DEAE sepharose (2.6x90cm). The unbound IgG fraction was adsorbed on a CM sepharose column (2.6x90cm) equilibrated with the same buffer. Following extensive washing (10 bed volumes), the IgG fraction was eluted with 0.5M NaCl in 10mM phosphate buffer pH 7.5

After dialysis against 10mM phosphate buffer pH 7.5 the IgG fraction was subjected to preparative isoelectric focusing in a flat bed Ultradex gel (LKB Ltd) using ampholytes of pI 8.0–pI 10.5 run at 10 watts for 14 hours at 4°C. The gel was divided into 30 fractions and the IgG was eluted with 2ml of distilled water. Fractions containing the relevant band were identified by analytical isoelectric focusing and silver staining.

The purified IgGs were assayed by ELISA using monoclonal antibodies to the four IgG subclasses (Seward Labs).

Anti-idiotypic antibodies to these isolated IgGs were raised in rabbits. Typically, 100μg of IgG emulsified with complete Freund's adjuvant was injected intradermally in a rabbit. Following a booster injection two months later, the animals were bled. Each antiserum was adsorbed three times with a sepharose IgG immunosorbent to remove antibodies specific for common IgG H and L chain determinants. The specificity of the adsorbed antisera was measured by ELISA, on a range of purified IgG preparations.

Results

The purity of the isolated IgGs is illustrated in Figure 1. The fractionation procedure yielded 2.6mg of IgG (FS), 1.5mg of IgG (MS) and 0.5mg of IgG (BB). The purified IgG bands were found only to react with monoclonal anti-IgG_1 subclass antibodies by ELISA.

Each rabbit produced antibodies which reacted with the immunizing band, however, following extensive adsorption with normal human IgG, all of the antibody raised against IgG (BB) was removed.

The anti-IgG (MS) antiserum was rendered specific for IgG (MS) by the adsorption procedure, while the anti-IgG (FS) antiserum showed substantial reactivity with IgG (FS), (BB) and (MS) bands by ELISA (Figure 2). A further study of ELISA of the specificity of the anti-IgG (FS) antiserum using purified paraproteins confirmed that the antiserum reacted solely with the isolated cationic IgGs (Table I).

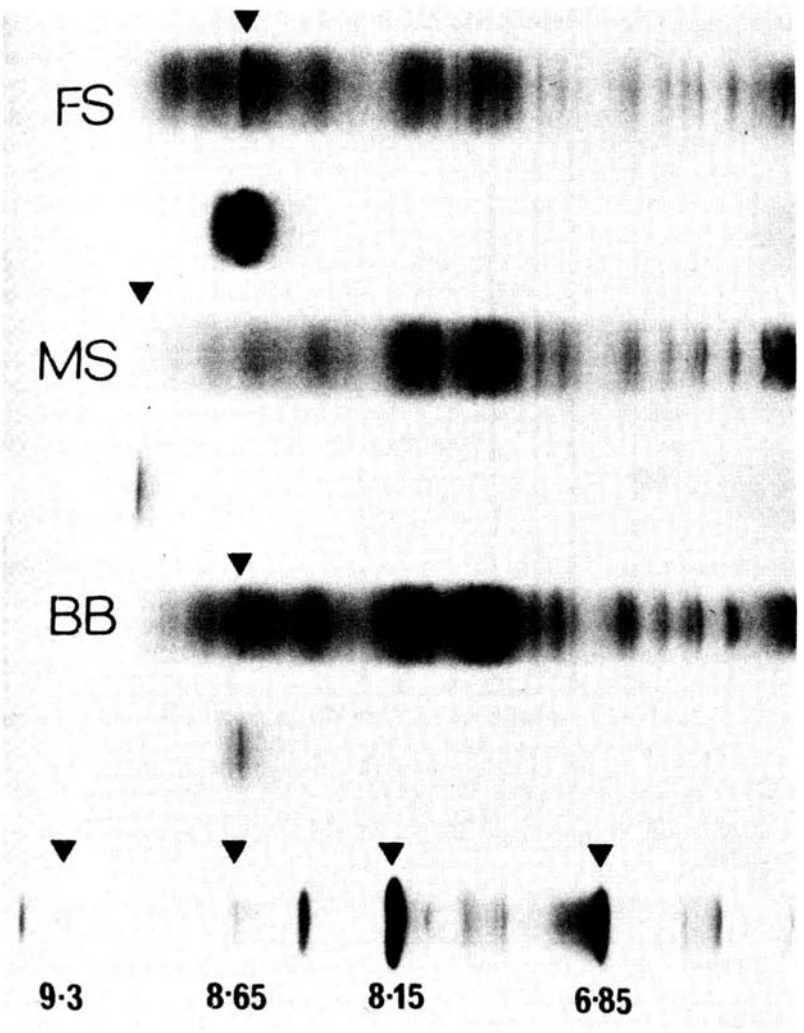

Figure 1. Analytical isoelectric focusing of serum and purified IgG bands from three patients (FS, MS and BB) with membranous nephropathy. The isoelectric points of marker proteins are shown in the lower track

Discussion

Abnormal cationic IgGs of pI 8.6, 8.6 and 9.0 in patients BB, FS and MS respectively have been purified from serum obtained from those patients at the time of declining renal function. All three IgG bands were of the IgG_1 subclass, a subclass known to fix complement efficiently and commonly identified in the kidney biopsies of patients with membranous nephropathy [5].

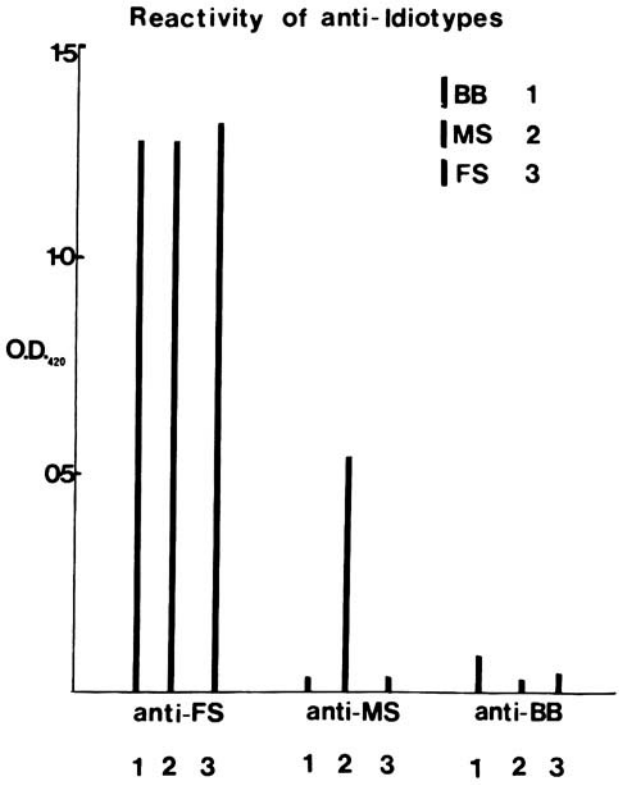

Figure 2. Reactivity of antisera to IgG (FS, BB and MS) after adsorption with normal human IgG

TABLE I. Specificity of anti-IgG (FS) antiserum by ELISA

Protein	pI	Reactivity with anti-IgG (FS)
FS	8.6	+
BB	8.6	+
MS	9.0	+
IgG_1 lambda	8.5	–
IgG_1 lambda	8.5	–
IgG_1 kappa	8.6	–
IgG_2 kappa	8.0	–
IgG_3 kappa	7.5	–
IgG_4 kappa	6.5	–
IgG (Cohn Fr II)	6–9.0	–
IgG (DEAE)	7.5–9.0	–
IgM	–	–

The role of these cationic IgGs in the disease process is uncertain. In order to implicate these serum IgG bands in the pathogenesis of membranous nephropathy it would be necessary to identify these antibody molecules within glomerular immune deposits.

Normally an antibody can be characterized by specificity for antigen. However, in the absence of antigen specificity, the presence of unique epitopes located within the antigen binding site (private idiotype) or cross reacting epitopes (public idiotype) related to the V region framework amino acid sequence, offers a potential method of characterizing the antibody. Several types of autoantibodies of known specificity, e.g. anti-DNA, anti-thyroglobulin and rheumatoid factors occurring in patients with autoimmune diseases have been shown to possess such cross reacting idiotypes [6].

Therefore the anti-IgG (FS) antibody may allow identification of these IgGs in biopsy specimens. The cross reacting epitope may be a marker of disease activity in patients with membranous nephropathy.

Acknowledgments

We wish to acknowledge financial support from the North West Kidney Research Association.

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