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ANTI-BOVINE SERUM ALBUMIN ANTIBODY IDIOTYPES IN PATIENTS WITH IgA NEPHROPATHY

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Summary

We have isolated idiotypic antibodies against a common diet antigen (bovine serum albumin, BSA) from a patient with IgA nephropathy. A heterologous anti-idiotype was also obtained from rabbits and used to study shared idiotypes in sera from patients with IgA nephropathy by ELISA. Serum levels of this idiotype were higher than normal in 14 of 28 patients studied. These shared idiotypes were significantly increased in serum from patients with IgA-complexes by Raji assay. Concordance was found between idiotype levels and haematuria. These results suggest that in patients with IgA nephropathy there is a restriction in the immunological response repertoire. In addition, idiotypes might be involved in the immune complexes of these patients and therefore be implicated in the pathogenesis of this disease.

Introduction

Patients with IgA nephropathy have very often elevated levels of antibodies against a large array of exogenous antigens (bacterial or dietary antigens), probably indicating a defect in the antigen exclusion at the mucosal level and/or an abnormality in the regulation of IgA [review in 1]. In a previous paper we have shown that in these patients IgA immune complexes and complexes containing antibodies against diet antigens present fluctuations after the ingestion of a large amount of protein [2]. This fact suggested that anti-idiotypic antibodies might participate in the in vivo formation of immune complexes, competing with the dietary antigens for the binding sites of the antibody against the idiotype. Recently we have isolated from a patient with IgA nephropathy, anti-bovine serum albumin (BSA) idiotype and obtained a polyclonal anti-idiotype in rabbits. By using this purified antiserum we have looked for the presence of shared idiotypes in the serum of these patients, as well as the correlation between these idiotypes and the clinical activity.

Material and methods

From a patient with IgA nephropathy and high serum levels of anti-BSA antibodies we have obtained the IgG and IgA fractions by a DE-52 cellulose column chromatography [3] and rendered them BSA specific by absorption on an AH-Sepharose 4B with BSA coupled using glutaraldehyde. After elution the anti-BSA IgG antibodies were used to obtain anti-idiotypic sera following rabbit immunization. The serum from these animals were rendered idiotype specific by extensive absorption on a human γ-globulin Sepharose column (in preparation). The fact that the binding of the anti-BSA antibody idiotype to its anti-idiotype could be inhibited by the antigen is in favour that the polyclonal anti-idiotypes are directed against the antigen-binding site on BSA antibodies. The heterologous anti-idiotype antibody was employed to measure the levels of anti-BSA antibody idiotypes in 28 patients with IgA nephropathy and 10 normal controls. Sera from these subjects were stored in aliquots at -70°C until tested.

Cross reactivity studies were performed by ELISA assay as follows: wells of polystyrene microtitre plates were coated with sera from patients and controls, previously diluted 1/100 with 0.01M carbonate-bicarbonate buffer pH 9.6, and incubated overnight at 4°C. After washing with PBS-Tween-Azide 800ng/well of the hetero antibody to anti-BSA IgG were added and placed for two hours at room temperature in a moist chamber. Unbound material was removed by washing and a dilution 1/500 of goat anti-rabbit immunoglobulin labelled with alkaline phosphatase (AHAB, Scarborough, ME, USA) was added and again incubated. The amount of alkaline phosphatase bound in the well was determined by measuring the hydrolysis of p-nitrophenyl phosphate (NPP). All experiments were performed in duplicate.

The presence of IgA and IgG immune complexes by Raji cell assay, as well as the statistical analysis, were performed as published [4].

Results

Fourteen out of 28 (50%) patients with IgA nephropathy had higher serum levels of idiotypes against BSA than normal subjects (Figure 1). Moreover, idiotype levels were also significantly higher in sera with IgA immune complexes (72%) than in those without these complexes (10%) (Figure 2). By contrast, no correlation was found between the levels of idiotypes and the presence or absence of IgG immune complexes (data not shown).

Patients with haematuria had higher levels of idiotypes against BSA than those without haematuria (Figure 3).

Discussion

In patients with IgA nephropathy the fluctuations of IgA immune complexes and complexes containing antibodies against dietary antigens after the ingestion of a large amount of protein suggested that antibodies against food antigens

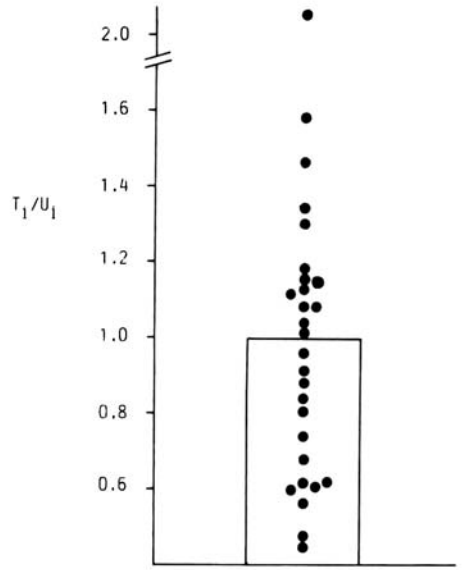

Figure 1. Levels of anti-BSA antibody idiotypes in serum from patients with IgA nephropathy (●). The mean of the duplicate values of the test sera (Ti) was divided by Ui (upper 95% confidence limit) construction as described in [4]. If the value T_i/U_i was ⩾1, the test serum was judged abnormal

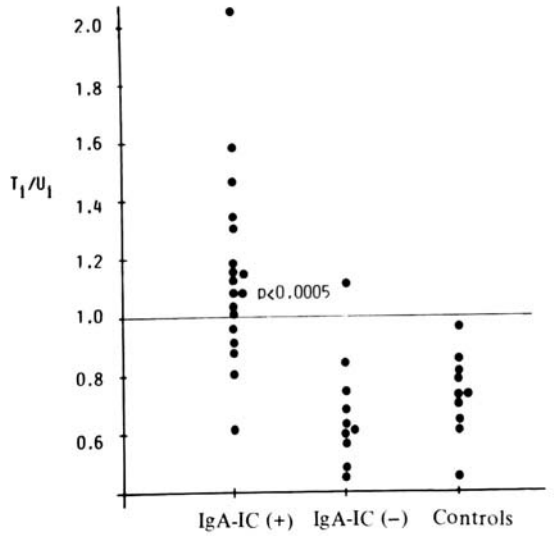

Figure 2. Levels of anti-BSA antibody idiotypes in serum from patients with IgA nephropathy

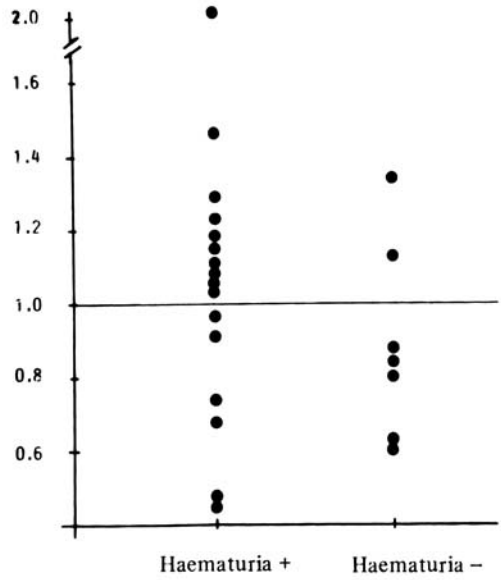

Figure 3. Anti-BSA antibody idiotypes and activity of the disease in patients with IgA nephropathy

and anti-idiotype antibodies could participate in the in vivo immune complexes formation, as occur in patients with selective IgA deficiency. Recently, from the serum of a patient with IgA nephropathy and high serum levels of anti-BSA antibodies, we have isolated the idiotypes and the respective auto-anti-idiotypes. The heterologous antibodies against the anti-BSA antibody idiotypes were obtained from immunized rabbits and used to study serum idiotypes.

The present study demonstrates the presence of shared idiotypes in 50 per cent of serum samples from a group of 24 unrelated patients with IgA nephropathy. Most often shared idiotypes have been found on several auto-antibodies, including rheumatoid factors, anti-thyroglobulin antibodies, antibodies to acetylcholine receptors, cold agglutinins and anti-DNA antibodies. However, related idiotypes are not peculiar to autoantibodies and are also found in conventional antibodies. Therefore, the production of antibodies with shared idiotypes appears to be a general property of the immune system, probably indicating restrictions in the repertoire of variable region immunoglobulin genes [5].

The fact that 13 out of 18 patients having IgA immune complexes, measured by Raji cell assay, have simultaneously high serum levels of anti-BSA antibody idiotypes, suggests indirectly that these idiotypes might be involved in the circulating immune complexes. These data are also consistent with the finding of high serum levels of idiotypes in the presence of haematuria (10 out of 16 patients). Similar results have been published recently in patients with systemic lupus erythematosus, in which a concordance was found between anti-DNA

antibody idiotypes and clinical activity in eight of the 12 patients studied [6]. Even in some cases the clinical status was reflected better by the idiotype levels than by the levels of anti-double stranded DNA antibodies. In this sense, deposition of idiotype-anti-idiotype immune complexes in renal glomeruli have been described in experimental situations [7]. From our results we do not mean to implicate the anti-dietary antibodies as playing a pathogenetic role in IgA nephropathy, although it has been suggested in other nephritis [8]. There is another possibility: antibody molecules with different antigen binding specifities have been found to share idiotypes [9]. In lupus-prone MRL-lpr/lpr mice, about 50 per cent of the anti-DNA antibodies in the serum of these animals share a common idiotype, although only about 20 per cent of the idiotype-positive antibodies bind to DNA, the specificities of the remaining 80 per cent being unknown [10]. Therefore, it is possible that the idiotypes detected in our patients could also be directed to other more pathogenic antigens.

In summary, these preliminary results show that patients with IgA nephropathy have shared idiotypes, probably indicating a restriction in the immunological response repertoire, although its physiopathological implications warrant further studies.

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References

1 Egido J, Sancho J, Blasco R et al. *Nephrology Vol I. Proc IXth Int Congr Nephrol, Los Angeles.* 1984: 652–664
2 Sancho J, Egido J, Rivera F, Hernando L. *Clin Exp Immunol 1983; 54:* 194
3 Egido J, Sancho J, Hernando P et al. *Contrib Nephrol 1984; 40:* 80
4 Egido J, Sancho J, Rivera F, Hernando L. *Nephron 1984; 36:* 59
5 Seidman JG, Leder A, Nay M et al. *Science 1978; 202:* 11
6 Isenberg DA, Shoenfeld Y, Madaio MP et al. *Lancet 1984; ii:* 417
7 Goldman M, Rose LM, Hochmann A, Lambert PH. *J Exp Med 1982; 155:* 1385
8 Van der Woude FJ, Hoedemaeker PHJ, Van der Giessen M et al. *Clin Exp Immunol 1983; 51:* 587
9 Hiernaux J, Bona CA. *Proc Natl Acad Sci USA 1982; 79:* 1616
10 Rauch J, Murphy E, Roth JN et al. *J Immunol 1982; 129:* 236