Proc EDTA–ERA (1985) Vol 22

ABNORMAL EXPRESSION OF THE GOODPASTURE ANTIGEN IN PATIENTS WITH ALPORT'S SYNDROME

C O S Savage, C D Pusey, *M Kershaw, S Cashman, †P Harrison, +B Hartley, †D R Turner, +J S Cameron, †D J Evans, C M Lockwood

*Royal Postgraduate Medical School, London, *Kingston Hospital, Surrey, †Southmead Hospital, Bristol, +Guy's Hospital, London, United Kingdom*

Summary

A mouse monoclonal antibody which recognizes that determinant in the glomerular basement membrane against which autoantibodies are directed in Goodpasture's syndrome, was used in indirect immunofluorescence studies to demonstrate an alteration or reduction in quantity of the Goodpasture autoantigen in 10 patients with Alport's syndrome. Studies of normal tissue show that the autoantigen is present in sites other than the kidney, including membranes within the cochlea and internal limiting membrane of the retina, suggesting that a biochemical defect of certain basement membranes, involving the Goodpasture antigen, may underlie the pattern of organ damage in Alport's syndrome.

Introduction

Alport's syndrome is an inherited condition [1], associated with ultrastructural abnormalities of basement membranes within kidney, cochlea and eye [2–4], although the biochemical defect has not been defined. There are reports that autoantibodies to the glomerular basement membrane (GBM) from patients with Goodpasture's syndrome do not bind to the GBM as Alport's syndrome [5], but others have been unable to confirm this finding [6]. We have developed a mouse monoclonal antibody (MCA-P1) which recognizes the Goodpasture antigen and have used this reagent to investigate basement membrane structure in Alport's syndrome.

Materials and methods

Patients Ten patients (9 male, 1 female) with Alport's syndrome who fulfilled three diagnostic criteria were studied: 1) the presence of haematuria; 2) high-tone sensori-neural deafness or specific eye lesions (macular flecks or lenticonus) in the patient, and/or a family history of renal disease or deafness, and 3) diffuse

thickening and splitting of GBM on electron microscopy consistent with Alport's syndrome.

Four patients (2 male, 2 female) were studied in whom the diagnosis of Alport's syndrome was considered equivocal on the basis of the electron microscopy appearance. Two patients (cases 11 and 12) had clinical histories suggestive of Alport's syndrome, but their GBM only showed patchy areas of thickening and splitting on electron microscopy. Case 13 had microscopic haematuria and a normal GBM appearance on electron microscopy, but her son had Alport's syndrome. Case 14 has a clinical diagnosis of benign familial haematuria and a diffusely thin GBM on electron microscopy.

Kidney biopsy material Percutaneous renal biopsy material was stored in liquid nitrogen for indirect immunofluorescence studies. Surgical and post mortem specimens of fresh normal kidney, and biopsy material from 15 patients with other forms of glomerulonephritis were processed and stored in a similar way.

Monoclonal antibodies The production and characterization of MCA-P1 has been described in detail elsewhere [7]. Briefly, it binds in a linear pattern to normal GBM, distal TBM and Bowman's capsule; the same pattern is seen with anti-GBM antibodies eluted from kidneys of patients with anti-GBM nephritis. Competition between MCA-P1 and autoantibodies in patients' sera for autoantigenic determinants in GBM has been demonstrated using radioimmunoassay and Western blotting (Dash et al, unpublished). MCA-P1 also binds to normal basement membranes of alveoli, choroid plexus of the brain, lens capsule and choroid of the eye and membranes within the cochlea.

An irrelevant mouse monoclonal antibody, eluted anti-GBM antibodies from human kidney and eluate from normal human kidney, were used in control studies.

Indirect immunofluorescence Fixed sections of kidney, cut at 4–5μ on a cryostat, were incubated with a 1:10 dilution of MCA-P1 hybridoma supernatant or neat eluted anti-GBM antibodies. Irrelevant mouse monoclonal and eluate from normal human kidney were used as negative controls. After extensive washing, bound antibody was detected using fluorescein-linked rabbit anti-mouse IgG or goat anti-human IgG (Miles). After further washing, sections were mounted in 90% glycerol in phosphate buffered saline and examined under a fluorescence microscope. Intensity of staining was scored on a 0–4+ scale by two observers (SC, MJK) who were unaware of the case histories.

Results

MCA-P1 and eluted anti-GBM antibodies bound strongly to normal GBM, distal TBM and Bowman's capsule in a linear pattern, although staining with eluted anti-GBM antibodies was less intense and background fluorescence was higher. A similar pattern and intensity of staining was observed with both reagents on kidney biopsies taken from patients with minimal change, membranous and

TABLE I. Comparative intensity of staining of GBM and TBM with monoclonal antibody MCA-P1 and eluted anti-GBM antibodies (GPE) in the 14 patients studied

Patient	Binding to GBM		Binding to TBM	
	MCA-P1	GPE	MCA-P1	GPE
1	2+	+	±	0
2	0	0	0	0
3	+	+	0	0
4	+	+	0	0
5	0	0	0	0
6	+	+	+	+
7	+	+	+	–
8	0	0	0	0
9	0	0	0	0
10	2+	2+	2+	2+
11	4+	4+	+	+
12	4+	4+	+	+
13	4+	4+	4+	–
14	4+	4+	4+	4+

mesangio-capillary glomerulonephritis, systemic lupus erythematosus and diabetes mellitus. However, there was reduced or absent binding of MCA-P1 and eluted anti-GBM antibodies to GBM and distal TBM of the 10 patients with Alport's syndrome (Table I). In cases 11 and 12 there was normal staining of GBM but reduced binding to TBM, and in cases 13 and 14 the binding was normal.

There was no correlation between degree of GBM staining and quantity of proteinuria, extent of renal impairment at time of biopsy or thickness of GBM on electron microscopy. However, the four patients with absent binding to GBM were less than seven years of age and were male, whilst the six patients with reduced binding presented at varying ages between two and 31 years. Determination of whether patients with absent staining have more severe disease and thus present at a younger age must await further studies using more patients.

To exclude the possibility that antigenic determinants in biopsies from patients with Alport's syndrome were blocked by an exogenous inhibitor or were present but not immediately available for binding, the above studies were repeated after treating kidney sections with 0.1M glycine hydrochloride pH 2.2, 6M guanidine hydrochloride and 8M urea. There was no alteration in the observed binding pattern or intensity of staining.

Discussion

These studies involving MCA-P1, a reagent which specifically detects the Goodpasture antigen, suggest that there is a variable quantity of immunoreactive autoantigen in patients with Alport's syndrome. This supports the hypothesis

put forward by Spear in 1983 [8], that a genetic abnormality in Alport's syndrome may affect a structural gene which governs the composition of basement membranes within the glomerulus, inner ear and lens capsule. Indeed, ultrastructural abnormalities have been detected in GBM [2] and cochlear basement membrane [3] and are suspected to be present within basement membranes of the eye [4]. Abnormal antigenicity of GBM in Alport's syndrome has been suggested previously [5] and is further supported by the finding that the urine of children with Alport's syndrome contains an abnormal antigen [9].

The observation that MCA-P1 binds to basement membrane of normal cochlea, lens and retina, as well as to GBM, suggests that similar antigenic determinants are present in these basement membranes and so have the potential to be affected by similar genetically determined abnormalities. Confirmation of this hypothesis is awaited from studies using MCA-P1 on tissue other than kidney from patients with Alport's syndrome. The pattern of binding of MCA-P1 to GBM and TBM in cases 10–14 are intriguing. The normal binding of MCA-P1 to GBM and TBM in case 13 (the mother whose son was severely affected by Alport's syndrome who had absent binding of MCA-P1 to GBM) is compatible with X-linked transmission of the disease and variable lyonization of the affected X-chromosome in the mother [10].

The normal binding of MCA-P1 to GBM and TBM in case 14 (thought to have benign familial haematuria with a diffusely thin basement membrane) suggests that MCA-P1 may be able to differentiate Alport's syndrome from other types of inherited nephropathy.

Finally, the significance of the reduced binding of MCA-P1 to TBM but normal binding of MCA-P1 to GBM in cases 10 and 11 is not clear at present but may represent incomplete phenotypic expression of disease.

Further studies are now in progress using MCA-P1 to quantitate the excretion of urinary GBM antigen and using MCA-P1 to detect Goodpasture's antigen in kidney sections taken from paraffin blocks, allowing a larger number of patients to be studied retrospectively.

References

1 Alport AC. *Br Med J 1927; 1:* 504
2 Spear GS, Slusster RJ. *Am J Pathol 1972; 69:* 213
3 Weidauer H, Arnold W. *Arch Otorhinolaryngol 1975; 210:* 361
4 Govan JAA. *Br J Opthal 1983; 67:* 493
5 McCoy RC, Johnson KH, Stone WJ, Wilson CB. *Kidney Int 1982; 21:* 642
6 Habib R, Gubler M, Hinglais N et al. *Kidney Int 1982; 21:* S20
7 Pressey A, Pusey CD, Dash A et al. *Clin Exp Immunol 1983; 54:* 178
8 Spear GS. *Clin Nephrol 1973; 1:* 336
9 Lubec G, Shabbir C. *Lancet 1984; i:* 1119
10 Hasstedt SJ, Atkin CL. *Am J Hum Genet 1983; 35:* 1241