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Failure of two subsequent renal grafts by anti-GBM glomerulonephritis in Alport's syndrome: case report and review of the literature

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Abstract. We describe a patient with Alport's syndrome who developed severe crescentic glomerulonephritis after each of two successive transplantations, leading to accelerated graft failure on both occasions. This complication occurred in the 7th postoperative month for the first transplant and in the immediate postoperative period for the second. Immunopathological studies of the second transplant demonstrated that the glomerular lesions were mediated by antiglomerular basement membrane (GBM) antibodies displaying the same pattern of reactivity as the MCA-P1 monoclonal antibody directed against the Goodpasture antigen. This observation indicates that the anti-GBM immunization induced by renal transplantation in some patients with Alport's syndrome may be responsible for recurrent graft failure.

Key words: Alport's syndrome, glomerulonephritis – Glomerulonephritis, in Alport's syndrome

De novo antiglomerular basement membrane (GBM) glomerulonephritis has occasionally been reported after renal transplantation in patients with Alport's syndrome [2, 4, 5, 8, 9, 12, 13]. The defect of the GBM found in certain forms of the syndrome [3–6, 8, 9,] is thought to be involved in the pathogenesis of this rare complication. Indeed, it has been suggested that the introduction via the renal graft of GBM antigens lacking or abnormal in Alport's syndrome might lead to specific immunization against those antigens [8, 9].

Thus far, only four cases of retransplantation in patients with Alport's syndrome who developed crescentic glomerulonephritis on a first graft have been reported [5, 9, 12]. Only one case of crescentic glomerulonephritis was observed, despite the presence of anti-GBM antibodies in three patients. We report here on a patient in whom retransplantation failed owing to immediate recurrence of anti-GBM glomerulonephritis. Immunopathological studies indicate that the anti-GBM antibodies displayed the same pattern of reactivity as the MCA-P1 monoclonal antibody directed against the Goodpasture antigen.

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Case report

A 25-year-old Italian engineer, on chronic hemodialysis for Alport's syndrome since 1981, received his mother's kidney in June 1985. The diagnosis of Alport's syndrome had been made on the basis of: (1) a history of hematuria and proteinuria since childhood, (2) an audiogram demonstrating sensorineural hearing loss, and (3) a sister in end-stage renal failure. Post-transplantation immunosuppression consisted of cyclosporin A and prednisolone. An early rejection episode was successfully treated by pulses of methylprednisolone, and the patient was discharged on the 13th postoperative day (POD) with a serum creatinine concentration of 133 µmol/l. No complication was observed during the ensuing 7 months except for moderate

Table 1. Specificity of the anti-GBM antibodies: comparison with the MCA-P1 monoclonal antibody

	Reactivity with					
	Normal GBM	Alport's GBM		Lung	Cochlea	Skin
		1	2			
MCA-P1 MoAb[a]	+	–	+	+	+	–
Patient's antibodies[b]	+	–	+	+	+	–

[a] MCA-P1 monoclonal antibody recognizing Goodpasture antigen [12]
[b] Anti-GBM antibodies present in patient's serum or eluted from the kidney graft

arterial hypertension, which was easily controlled with metoprolol. In early February 1986, gross hematuria occurred and serum creatinine rose to 203 µmol/l. Simultaneously, the graft was found to be tender and enlarged. A putative diagnosis of acute rejection was made and the patient received pulses of methylprednisolone (1 g/day) for 5 consecutive days. Despite this therapy, graft function rapidly deteriorated and the transplant was excised at the end of February 1986. Microscopic examination of the graft revealed extensive crescentic glomerulonephritis without evidence of cellular or vascular rejection. Anti-GBM glomerulonephritis was suspected, but immunofluorescence stainings were not available and radioimmunoassay for anti-GBM antibodies [7] was persistently negative on repeated serum samples.

In April 1987, the patient was retransplanted with a cadaveric kidney, immunosuppression consisting of antilymphocyte globulin (Lymphoglobuline, Institut Mérieux, Lyon, France), azathioprine, and prednisolone. The graft functioned immediately, but hematuria was noted on POD 1 and persisted thereafter. Serum creatinine decreased from 1087 µmol/l on POD 2 to 477 µmol/l on POD 7. On POD 8, the patient developed high fever; urine output dropped and serum creatinine rose to 546 µmol/l. Five boluses of methylprednisolone were administered between POD 10 and 14, antilymphocyte globulin was stopped on POD 19, and cyclosporin A was started. Renal function continued to deteriorate and the patient developed extensive herpetic stomatitis, thrombocytopenia, and leukopenia, leading to azathioprine withdrawal on POD 21. The graft was removed on POD 29.

Immunopathological studies

Microscopic examination of the second graft (Fig. 1) demonstrated severe lesions of crescentic glomerulonephritis identical to those observed in the first transplant. Glomerular changes were associated with tubular necrosis, but neither vascular nor cellular rejection was observed. Immunofluorescence stainings revealed linear deposits of IgG in all glomerular segments escaping extracapillary proliferation (Fig. 2). IgG deposits were also present along some tubular basement membranes and Bowman's capsules. Stainings for IgM, IgA, and C3 were negative, whereas fibrin and fibrinogen were demonstrated in numerous crescentic glomerular lesions.

A retrospective serial study was conducted on eight sera obtained from the 2nd month following the first graft up to the 5th month after the second transplant. These serum samples were tested for anti-GBM antibodies by a specific solid-phase enzyme-linked immunosorbent assay (ELISA) using collagenase-solubilized human GBM [1]. As shown in Fig. 3, anti-GBM antibodies, which were absent in the first serum, were present when hematuria occurred 7 months after the first graft, and they persisted up to the 5th month after the second transplant. These data were confirmed by indirect immunofluorescence, which further demonstrated that the anti-GBM antibodies failed to react with an Alport's GBM lacking the capacity to bind the MCA-PI monoclonal antibody, while they stained another Alport's GBM reacting with this monoclonal antibody [10]. An acid eluate from the second graft was prepared as follows. A fragment obtained after explantation was minced, washed thoroughly in phosphate-buffered saline at pH 7.4, and then incubated for 30 min at 37°C in citrate buffer 0.02% pH 3.2. After centrifugation the supernatant was dialyzed against phosphate-buffered saline and the immunoglobulin fraction of the eluate was concentrated by precipitation with 50% ammonium sulfate. By indirect immunofluorescence, the anti-GBM activity of this eluate was found to be qualitatively similar to that of the serum. In addition, indirect immunoperoxidase stainings indicated that the eluate also reacted with the basement membranes of normal lung and cochlea but not of normal skin. The similarities between the reactivity profiles of the patient's anti-GBM antibodies and of the MCA-PI monoclonal antibody are outlined in Table 1.

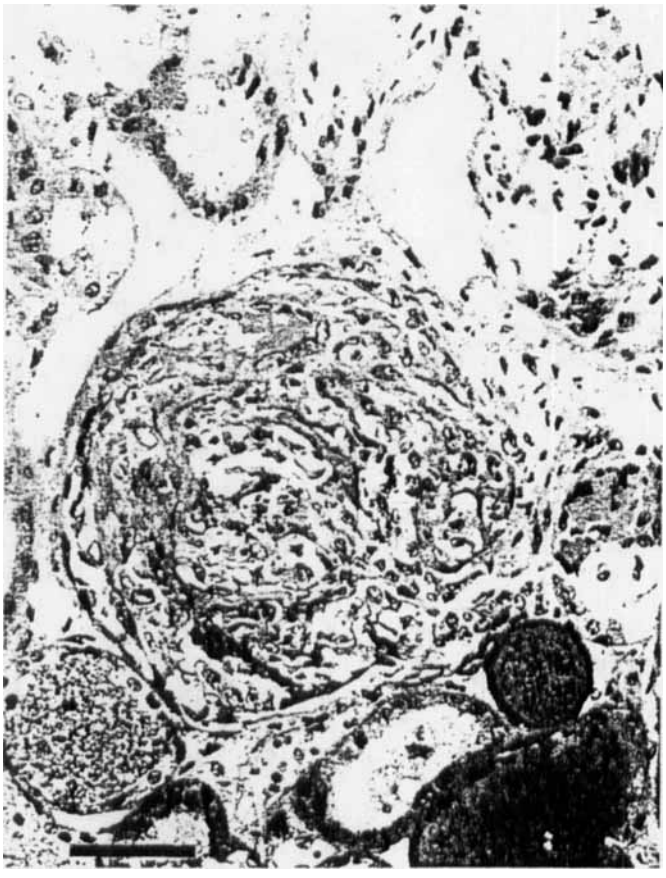

Fig. 1. Crescentic glomerulonephritis on the second transplant (*bar* equals 50 µm)

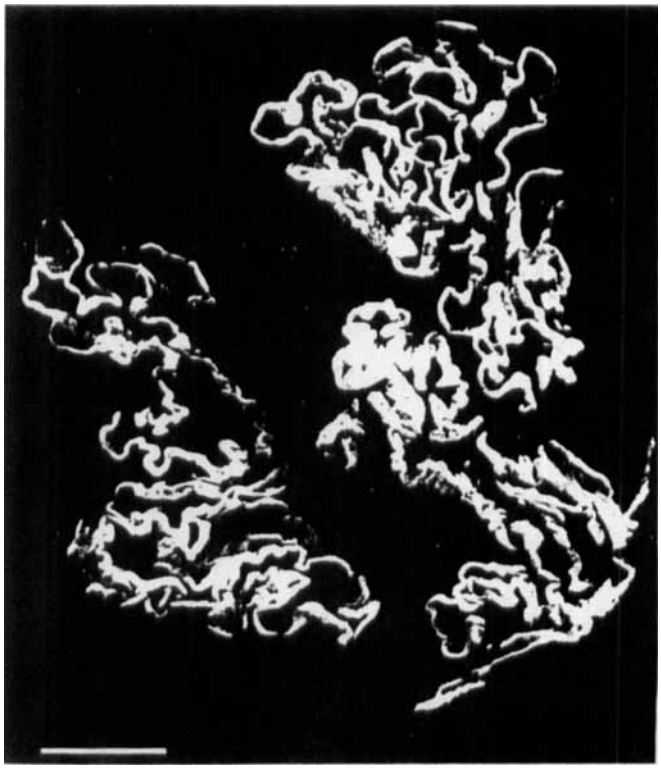

Fig. 2. Immunofluorescence staining for IgG showing linear deposits along capillary walls in the second transplant (*bar* equals 25 µm)

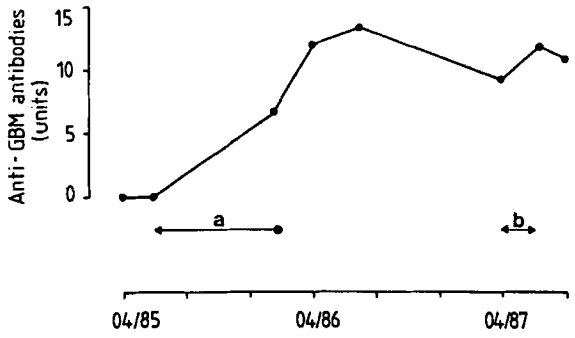

Fig. 3. Evolution of anti-GBM antibody levels determined by ELISA (*a*, first graft; *b*, second graft)

Discussion

This observation confirms the notion that some patients with Alport's syndrome are prone to develop anti-GBM crescentic glomerulonephritis after transplantation. Thus far, this complication has been reported on nine occasions in the literature [2, 4, 5, 8, 9, 12, 13]. The main data regarding these cases and our patient are summarized in Table 2. All patients were male, ranging in age from 14 to 34 years. Grafts were retrieved from related living donors in five cases. Immunosuppressive therapy consisted of currently used drugs, including cyclosporin A. Hematuria, the hallmark of glomerulonephritis, occurred between 1 and 22 months post-transplant, except in our patient, where it immediately followed the second transplantation. The final outcome of the graft was unfavorable in all cases, but the time interval between the onset of glomerulonephritis and explantation was highly variable (1–21 months).

Five Alport's patients who developed crescentic glomerulonephritis on a first graft were retransplanted. In the first patient (patient 2, Table 2), anti-GBM antibody was detected in the serum and graft biopsy demonstrated linear deposits of IgG along GBM, but the graft was functioning normally 2 years post-transplant and anti-GBM antibody was undetectable in serum at that time. In the second patient (patient 3), no biopsy was obtained, but anti-GBM antibody was not detected in serum and the graft was reported to be functioning normally in the 5th year. In the third patient (patient 6), early graft biopsy demonstrated linear IgG deposits along the GBM and anti-GBM antibody was present in serum; however, glomerulonephritis did not develop and early graft failure was due to acute cellular rejection. The fourth patient (patient 8) developed crescentic anti-GBM glomerulonephritis on the second transplant, which was lost after 3 months despite plasma exchanges and cytotoxic therapy. Our own observation (patient 9) indicates that recurrence of crescentic glomerulonephritis may even be more rapid, leading to early graft loss despite high doses of corticosteroids. Thus, the consequences of post-transplant anti-GBM immunization on the outcome of a second graft vary from individual to individual, the reasons for these differences being unknown.

Our immunopathological data confirm that the anti-GBM antibodies elicited by renal transplantation in Al-

Table 2. Patients with crescentic glomerulonephritis after transplantation for Alport's syndrome. Review of the literature, including present case. D, Dialysis; Tx, transplantation; GN, glomerulonephritis; Expl, explantation; M, male; CAD, cadaver; LRD, living related donor; ND, not determined; AZA, azathioprine; PRED, prednisone or prednisolone; CyA, cyclosporin; ALG, antilymphocyte globulin

Patient	Author [reference]	Sex	Age (years)		Graft origin	Immunosuppression	Interval (months) from Tx to		Anti-GBM antibody		Outcome
			D	Tx			GN	Expl	Graft	Serum	
1	McCoy [8]	M	14	15	CAD	AZA/PRED	5	ND	+	+	Back to D
2	Milliner [9]	M	34	34	LRD	AZA/PRED	9	14	+	+	Re-Tx: anti-GBM antibody in graft and serum; normal function at 2 years
3	Milliner [9]	M	24	27	CAD	AZA/PRED	22	43	+	0	Re-Tx: anti-GBM antibody absent in serum; normal function at 5 years
4	Teruel [13]	M	15	24	CAD	AZA/PRED	6	7	+	+	Back to D
5	Shah [12]	M	19	19	LRD	AZA/CyA/PRED	18	ND	+	+	Back to D
6	Shah [12]	M	20	20	LRD	AZA/CyA/PRED	18	ND	+	+	Re-Tx: anti-GBM antibody in graft and serum without GN; early failure due to rejection
7	Fleming [2]	M	17	32	CAD	CyA	9	10	+	+	Back to D
8	Kashtan [4,5]	M	ND	a) 20	LRD	ND	1	ND	+	+	Re-Tx: see b)
				b) 23	CAD	ND	3	ND	+	+	Back to D
9	Present case	M	21	a) 25	LRD	CyA/PRED	7	8	ND	+	Re-Tx: see b)
				b) 27	CAD	ALG/AZA/PRED	0	1	+	+	Back to D

port's syndrome are directed against epitopes similar or closely related to those recognized by the antibodies of patients with Goodpasture's syndrome [8]. Indeed, our patient's antibodies displayed the same pattern of reactivity as the monoclonal MCA-PI, which was found by Western blotting to share the specificity of Goodpasture antibodies [10]. This monoclonal antibody usually does not react with the GBM of Alport's kidneys, which indicates that the antigens lacking or abnormal in Alport's GBM are closely related to those involved in Goodpasture's syndrome [11]. The anti-GBM response elicited by renal transplantation in some Alport's patients could, therefore, be due to the introduction via the transplant of antigens to which the immune system has not been tolerized. The reasons why only a minority of Alport's patients develop this complication [1] could be related to the mode of presentation of the GBM antigens to the immune system or to genetic factors governing immune responsiveness. One should also bear in mind the differences in sensitivity and specificity of the various anti-GBM assays, something which may explain the failure to detect some anti-GBM responses, as was initially the case in our patient.

In conclusion, anti-GBM immunization may occasionally jeopardize the outcome of renal transplantation in Alport's syndrome. Further studies are required to define the characteristics of the small number of patients prone to develop this complication.

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