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Antineutrophil cytoplasm antibody in crescentic glomerulonephritis

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SUMMARY Antineutrophil cytoplasm antibody (ANCA) has been reported in the sera of adults with Wegener's granulomatosis and microscopic polyarteritis, but this phenomenon has not so far been described in children. We report three children with crescentic glomerulonephritis in whom ANCA concentrations were raised at presentation. Two cases were idiopathic, and the other later developed features of Wegener's granulomatosis. In all three, plasma exchange and immunosuppression removed ANCA, but in only one case was there clinical improvement. This child later developed classical nasal lesions of Wegener's granulomatosis associated with a decline in renal function and a rise in ANCA. Plasma exchange and immunosuppression again produced a good clinical response and removed ANCA. This suggests that ANCA is either a marker of disease activity, or is involved in the pathogenesis of disease.

Crescentic glomerulonephritis is characterised by intraglomerular extracapillary cellular proliferation and is a relatively rare pathological finding in childhood. It is often associated with the clinical picture of rapidly progressive renal failure. It may be idiopathic or associated with other diseases such as post-streptococcal glomerulonephritis, infective endocarditis, IgA nephropathy, Goodpasture's syndrome, membranoproliferative glomerulonephritis, systemic lupus erythematosus, Henoch-Schönlein purpura, and other systemic vasculitides. The pathogenesis of the histological lesion is incompletely understood.[1]

Microscopic polyarteritis and Wegener's granulomatosis are rare causes of crescentic glomerulonephritis in childhood. Circulating antineutrophil cytoplasm antibody (ANCA) has been described in these disorders in adults,[2–6] but not so far in children. We describe three children with crescentic glomerulonephritis in whom high concentrations of ANCA were detected both by indirect immunofluorescence by overlaying sera on normal neutrophils and by a solid phase radioimmunoassay.

Case reports

Case 1. An 11 year old girl presented in acute renal failure after a one week history of vomiting, abdominal pain, and haematuria. She was oliguric, normotensive, and her plasma creatinine concentration was 603 μmol/l. Despite having no clinical or bacteriological evidence of a recent streptococcal throat infection, she had a raised antistreptolysin O titre of 1200 U/ml (upper limit of normal 400 U/ml), a raised anti-DNAase B titre 600–800 U/ml (upper limit of normal 450 U/ml), and a low C3 concentration of 60% of reference normal serum (normal range 86–118%), suggesting a diagnosis of post-streptococcal glomerulonephritis. She responded to diuretic treatment, but one week after admission her plasma creatinine concentration was still raised at 530 μmol/l, and a renal biopsy was performed. The biopsy specimen contained nine glomeruli, all of which had large cellular crescents and hypercellular tufts (fig 1). Immunofluorescence showed the presence of fibrin in the crescents, but no other immunoreactants were detected. Circulating ANCA was shown in her serum by indirect immunofluorescence on normal neutrophils (fig 2), and confirmed by solid phase radioimmunoassay at a titre of 54% of a positive control (upper limit of normal range 15%).

In view of the severity of the histological changes she was treated with prednisolone 2 mg/kg/day, cyclophosphamide 3 mg/kg/day, and a course of daily 4000 ml plasma exchanges for five days using

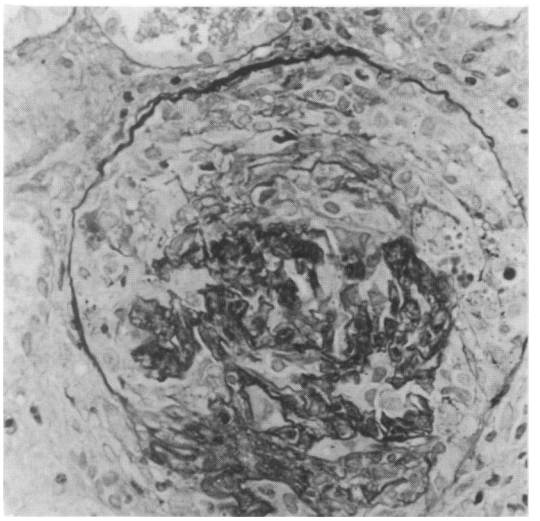

Fig 1 *High power section from the renal biopsy specimen of case 1 showing a glomerulus with a large cellular crescent.*

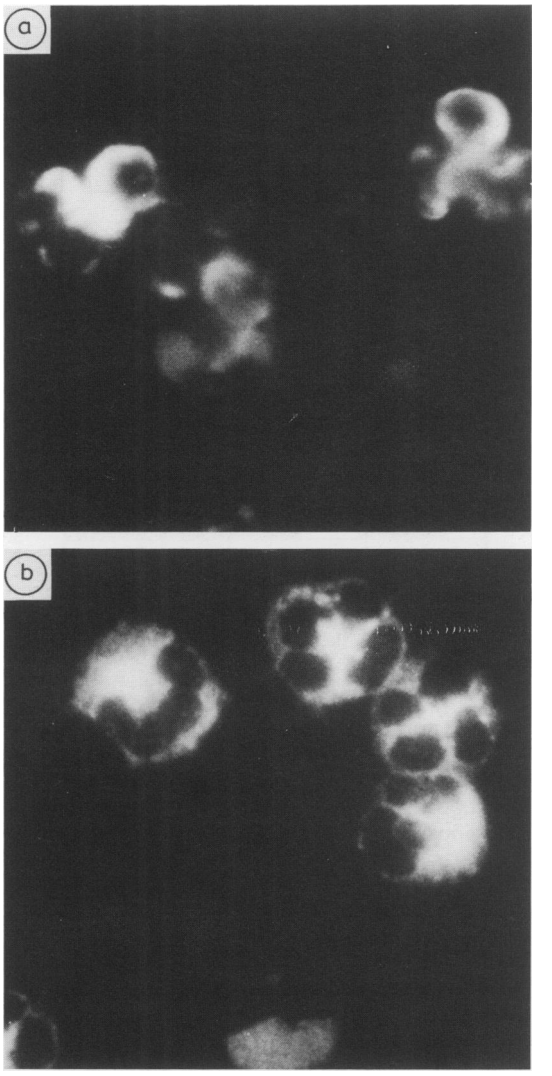

Fig 2 *Detection of ANCA by indirect immunofluorescence. Normal neutrophils are incubated with test serum (diluted 1/8), followed by fluorescein-isothiocyanate-conjugated rabbit antihuman IgG. (a) Serum from case 1: (b) serum from an adult case of Wegener's granulomatosis that has been proved on biopsy. Both show fluorescence of neutrophil cytoplasm.*

purified protein fraction as replacement with fresh frozen plasma for the last 300 ml of each exchange. After this her urine output increased and her plasma creatinine concentration fell to 120 μmol/l. One week later she was discharged home on a reducing dose of immunosuppressives. At this time her ANCA was 11% of positive control (within the normal range), and her glomerular filtration rate was 118 ml/min/1·73 m^2 surface area.

Four months later her glomerular filtration rate had fallen to 70 ml/min/1·73 m^2 surface area, and she developed nasal lesions that were histologically compatible with Wegener's granulomatosis. ANCA had risen to 60% of positive control. Another course of five plasma exchanges combined with cyclophosphamide and steroids again reduced the ANCA concentration to 9% of positive control and her glomerular filtration rate increased to 99 ml/min/1·73 m^2 surface area. One year later she remains well on prednisolone 30 mg on alternate days and azathioprine 100 mg/day with no evidence of recurrence of respiratory tract disease. She has a glomerular filtration rate of 94 ml/min/1·73 m^2 surface area, and has an ANCA concentration measured by solid phase radioimmunoassay of <5% of positive control. Her clinical course and circulating ANCA concentrations are summarised in fig 3.

Case 2. A 12 year old girl presented with a grand mal convulsion after a six week history of vomiting, malaise, and headaches. She was found to be in acute renal failure with anuria, fluid overload, hypertension, and her plasma creatinine concentration was 1560 μmol/l. She was treated by peritoneal dialysis. Initial investigations, which included a normal renal ultrasound, failed to establish the diagnosis. Serum antistreptolysin O titre and anti-DNAase titre were normal, but C3 was 76% of

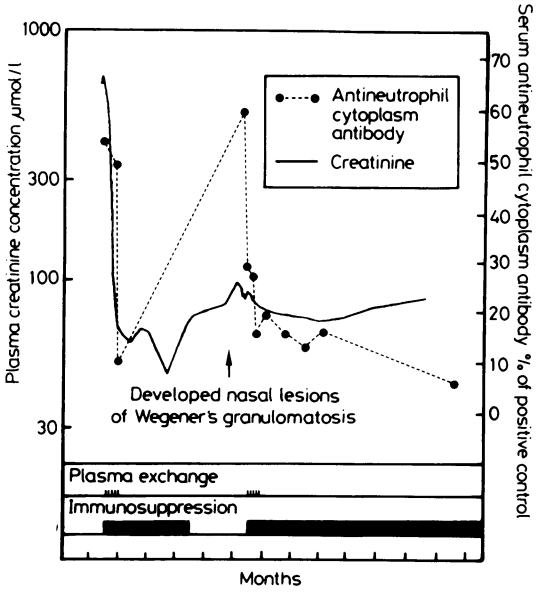

Fig 3 *Clinical course of case 1 showing plasma creatinine concentration and ANCA in relation to treatment.*

reference normal serum. She had circulating ANCA shown by indirect immunofluorescence, which when measured by solid phase radioimmunoassay was 41% of positive control. She remained anuric, and a renal biopsy specimen showed all of 60 glomeruli to be severely damaged with predominantly fibrous crescents and sclerosis and hyalinisation of the tufts. Immunofluorescence showed deposits of IgM, Clq, and C3 along the capillary loops.

A course of daily plasma exchanges for five days combined with prednisolone 2 mg/kg/day and cyclophosphamide 3 mg/kg/day reduced the circulating ANCA to 12% of positive control, but she remained anuric. She was entered into a programme for continuous ambulatory peritoneal dialysis but developed peritonitis due to Aspergillus and subsequently an acute meningoencephalopathy of unknown aetiology. This failed to respond to broad spectrum antimicrobial treatment, and despite intensive supportive treatment she died of rapidly progressive neurological disease. A postmortem examination was not performed.

Case 3. An 11 year old girl presented with acute renal failure six weeks after a 10 day illness consisting of sore throat, cough, fever, and haematuria. She was oliguric, fluid overloaded, and hypertensive, and her plasma creatinine concentration was 521 μmol/l. She was treated by peritoneal dialysis. Initial investigations showed an antistreptolysin O titre of 400–600 U/ml, an anti-DNAase B titre of 100–200 U/ml, and a normal C3 concentration of 112% of reference normal serum. She had circulating ANCA detectable by immunofluorescence, which measured by solid phase radioimmunoassay was 32% of positive control. A biopsy specimen showed 36 (95%) of 38 glomeruli to have predominantly fibrous crescents and advanced global sclerosis. Immunofluorescence showed scanty IgM and C3 deposition along capillary loops. Despite a course of daily plasma exchanges for five days combined with prednisolone 2 mg/kg/day and cyclophosphamide 3 mg/kg/day, which removed detectable ANCA, she showed only a transient improvement in renal function. She was entered into a renal replacement programme, ANCA subsequently remained undetectable, and she received a successful renal transplant 13 months after her initial presentation.

Methods

ANCA was detected by two methods:

(1) INDIRECT IMMUNOFLUORESCENCE ON NORMAL NEUTROPHILS

This technique has been described previously.[5] Normal neutrophils from healthy donors are isolated by density gradient centrifugation and alcohol-fixed on glass microscope slides. The slides are incubated for 1 hour at 4°C first with dilutions of test or control sera, and then with fluorescein-isothiocyanate-conjugated rabbit antihuman IgG. The slides are then examined under ultraviolet light, and samples are considered positive if most neutrophils show bright fluorescence in the cytoplasm (fig 2), and negative if no staining is present.

(2) SOLID PHASE RADIOIMMUNOASSAY

This technique has also been described previously.[5] Neutrophil antigen is prepared from normal donor neutrophils by sonication and centrifugation in sodium acetate (0·2 mol/l, pH 4·2), and the supernatant saved. Wells of plastic microtitre plates are coated with the antigen by incubating with the supernatant for 1 hour at 37°C. Dilutions of test or control sera are then incubated in the wells for 30 minutes at 37°C. IgG bound to the wells is detected by a treble-layer technique (mouse monoclonal antihuman IgG, then rabbit antimouse IgG, then ^{125}I-labelled goat antirabbit IgG), and measured by counting in a gamma counter. The results of this binding are expressed as a percentage of the binding seen for a standard positive serum from an adult case of Wegener's granulomatosis that has been proved on biopsy.

Discussion

Crescentic glomerulonephritis is a relatively rare condition in childhood.[1] The severe form can be defined as the presence of crescents in at least 80% of glomeruli and presents as rapidly progressive glomerulonephritis. Only 10 new cases of this severe form have been admitted to the renal unit of the Hospital for Sick Children, London since June 1985 when we started screening these patients for raised ANCA; during this same period we dialysed 171 children with other causes of acute renal failure. In addition to the three cases reported here, three cases were associated with Henoch-Schönlein syndrome, one with anti-glomerular basement membrane nephritis, one with post-streptococcal glomerulonephritis, and two cases were idiopathic. Only the three cases reported here, however, had raised ANCA at presentation. Although ANCA has been shown in the sera of adult patients with Wegener's granulomatosis and microscopic polyarteritis,[2–6] it has not previously been reported in children. In case 1, the diagnosis initially appeared to be post-streptococcal glomerulonephritis despite there being no clinical or microbiological evidence of a throat or skin infection. Four months later, however, she developed clinical symptoms which, supported by histological evidence, established the diagnosis as Wegener's granulomatosis. Cases 2 and 3 both appeared to be idiopathic at presentation, but in view of the raised ANCA and the experience from adult cases, might perhaps represent occult microscopic polyarteritis.

The significance of the presence of this antibody is unclear, but a role in the pathogenesis of vasculitis is suggested as its concentration has been shown in adults to decrease as clinical signs of disease activity improve.[6] In the three children described here, plasma exchange and immunosuppression resulted in an appreciable reduction in concentrations of circulating ANCA, but only in case 1 did this coincide with important clinical improvement. She had the shortest history, and in contrast with the others her renal biopsy specimen showed predominantly cellular crescents. When she later relapsed with deterioration of renal function and the development of the nasal lesions of Wegener's granulomatosis, her circulating ANCA concentration had again risen (to 60% of positive control), suggesting that the antibody had either a pathogenic role or was a marker of disease activity.

Circulating ANCA should be sought in children with crescentic glomerulonephritis, and when present may be of diagnostic value, pointing to Wegener's granulomatosis or microscopic polyarteritis. It may also be of value in monitoring the effectiveness of treatment in cases with a short duration of symptoms.

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