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Possible C1q bypass loop activation in the haemolytic uraemic syndrome

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SUMMARY

Ultrastructural and immunofluorescent microscopic studies were performed on renal tissue obtained from nine patients during the acute and convalescent phase of the haemolytic uraemic syndrome (HUS). All had glomerular deposits of IgM in the absence of circulating immune complexes. This was associated with deposition of Clq during the acute phase, and properdin and C3 during the convalescent phase. C4 was consistently absent. Since such a pattern of complement deposition does not fulfil criteria either for alternate or classical pathway activation, the possibility of Clq bypass loop activation by IgM is suggested.

INTRODUCTION

The haemolytic uraemic syndrome (HUS), characterized by acute renal failure, haemolytic anaemia and thrombocytopenia, was described by Gasser *et al.* (1955). The aetiology of this disease is unknown. Two basic pathogenic mechanisms have been proposed: a microangiopathic injury triggered by endotoxins leading to intravascular coagulation, as seen in the Shwartzman reaction, or an immune injury involving antigen–antibody complex formation. Similarities between the Shwartzman reaction and the HUS are many. In this reaction, endotoxins are believed to initiate the sequence of pathogenic events. However, attempts to demonstrate the presence of such circulating endotoxins using several methods have been unsuccessful (Van Wieringen, Monnens & Bakkeren, 1976).

Earlier immunofluorescence studies carried out on renal tissue in this syndrome failed to demonstrate immunoglobulin or complement glomerular deposits, thus arguing against an immunopathogenic type of injury (Gervais *et al.*, 1971; Habib *et al.*, 1969). More recently, however, positive immunofluorescence findings have been reported, describing glomerular deposits of IgM and complement (Cossio *et al.*, 1975; McCoy, Abramowsky & Krueger, 1974). Chronic glomerulonephritis associated with HUS has also been observed with immunoglobulin and C3 glomerular deposits (Cossio *et al.*, 1977).

This paper describes the immunofluorescent and histological findings in nine children with HUS admitted to our hospital over a period of 4 months. Glomerular as well as vascular deposits of IgM and complement components were present in all nine patients, suggesting immune-mediated injury in the glomeruli. The presence of both alternative and classical pathway complement components in the absence of C4 deposits suggested Clq bypass loop activation (May & Frank, 1973).

PATIENTS AND METHODS

Patients. During a period of 4 months in the summer of 1977, nine patients presenting with severe HUS were admitted and treated in our hospital.

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The mean age of these children was 3·75 years, the youngest being aged 5 months and the eldest 11 years. Distribution was equal for both sexes. No geographical factor could be identified; the affected children were living in areas scattered throughout the province of Quebec. The prodomal episode consisted of either gastro-intestinal disorders or upper respiratory tract infection preceding the acute phase of the syndrome by 6–10 days. Five patients presented with anuria of at least 24 hr duration. Three of these five patients had neurological involvement, two of whom eventually died, whilst a third exhibited only partial recuperation of renal function. All patients were treated by peritoneal dialysis, blood transfusions, dipyridamole (5·0 mg/kg) and acetylsalicyclic acid (60 mg/kg).

Renal tissue was obtained early in the course of the disease in three patients between day 5 and 12 of the acute phase, either at the time of autopsy in two patients or by open renal biopsy in one patient. In the other six patients, a percutaneous renal biopsy was performed between day 21 and 48 at a time when renal function had returned to normal.

Light microscopy. Renal tissue was fixed in Bouin's solution, embedded in paraffin, and cut into 2·0 μm sections. The sections were stained with Masson's trichrome, periodic acid silver methenamine and other selected stains.

Electron microscopy. Small pieces of renal tissue were fixed in 3% glutaraldehyde, in 0·1 M phosphate buffered saline and then post-fixed in 1% osmium tetroxide. They were embedded in Epon 812 and cut on a Reichart ultra-microtome. The sections were stained with uranyl acetate and lead hydroxide and studied with a Philips EM 201 electron microscope.

Immunofluorescence. The specimens were frozen in isopentane pre-cooled in liquid nitrogen and kept in a liquid nitrogen container. 4·0 μm sections were stained with fluorescein-conjugated anti-human IgG (Wellcome Reagents Ltd, Ontario, Canada), IgA, IgM, C3, fibrin (Hyland Laboratories) Clq, C4 (Behring Diagnostics, Montreal, Canada) and properdin (Atlantic Antibodies, Westbrook, Maine). The specificity of these antisera had been verified by immunoelectrophoresis. The slides were examined with a Zeiss fluorescent microscope.

Detection of immune complexes. Venous samples for immune complex detection were drawn during the first week of acute illness from six of the nine patients. Blood was allowed to clot at room temperature for 2 hr, serum was separated by centrifugation at 200 *g* for 15 min and stored in aliquots at −70°C until use. Clq-binding immune complexes were sought by the solid phase radioimmunoassay described by Hay *et al.* (1976). Rabbit anti-human IgG and IgM antisera were purified by immunoadsorbance on insolubilized Sepharose-cyanogen bromide human IgM and IgG columns, according to the method of Cuatrecasas, Wilchek & Anfinsen (1968). Both rabbit anti-human IgG and anti-IgM were labelled with ^{125}I according to the method of Hunter & Greenwood (1962) for use in the radioimmunoassay. Samples were run in duplicate and standards of aggregated human IgG, as well as sera drawn from patients with systemic lupus erythematosus prior to treatment were included as positive controls.

RESULTS

Light microscopy

Renal histological findings were similar to those previously described (Gervais *et al.*, 1971). At the acute stage, the predominant lesions were associated with renal intraglomerular thrombosis. In one of the patients who died, bilateral cortical necrosis with fibrinoid arteriolar necrosis was observed. Massive thrombi were noted in juxta-medullary glomeruli associated with tubular necrosis. In the other two patients, a major degree of capillary obstruction by thrombi was noted, but the most prominent finding was of mesangial oedema without significant proliferation. This oedema contributed to the obliteration of some capillary loops and to a contraction of Bowman's space. Clear subendothelial spaces, associated with the accumulation of fibrillar material, often gave the impression of a 'double contour' image.

On renal biopsies obtained late during the development of the disease, discrete thickening of the arteriolar walls was noted due to the medial accumulation of fibrillar material. Glomerular lesions appeared secondary to ischemia as indicated by endothelial cell swelling with obliteration of capillary loops. Interstitial lesions were also present, characterized by small foci of fibrosis and tubular atrophy and, in one patient, haemosiderin deposits were observed.

Electron microscopy

Electron microscopic findings were typical of thrombotic microangiopathic disease. In the acute phase, the arterioles were obstructed by platelets and polymorphonuclear leucocytes. Non-homogeneous dense deposits were found in the subendothelial spaces. Mesangial cells exhibited morphological alterations due to oedema as shown by structural changes of the endoplasmic reticulum and the presence of intracytoplasmic vacuoles. There was little proliferation and basement membranes were generally intact. On late biopsies, glomeruli were still retracted, the basement membranes had irregular outlines and electron dense material could be found in subendothelial spaces.

Immunofluorescence microscopy

Immunofluorescence studies performed on early biopsies showed granular deposits of IgM and Clq assuming the same distribution as fibrin along the arteriolar and glomerular capillary walls, as well as in the mesangium. Staining for IgG, IgA, C3, C4 and properdin was negative.

The same irregular deposits of fibrin and IgM could be found on tissue obtained from late biopsies, but the intensity of staining was less conspicuous. These deposits could also be found along the arteriolar walls and in the mesangium. However, only one of these biopsies was positive for Clq and the most striking observation made was of the presence of C3 deposits mainly along the arteriolar walls. Moreover, properdin was deposited in the same distribution as C3 in five out of six patients. No IgG, IgA or C4 could be detected. These immunoglobulin deposits could be readily eluded with citrate buffer (pH 3·2, 0·02 M). One patient biopsied on day 21 had deposits of Clq, C3, IgM and properdin (Table 1).

TABLE 1. Immunofluorescent microscopy

Patient	Biopsy (days after) admission	IgM	Clq	C3	Properdin	Fibrin
1	5	++	++	neg.	neg.	++
2	8	++	++	neg.	neg.	++
3	12	+++	++	neg.	neg.	+
4	21	+	+	+	+	+
5	33	++	neg.	+	+	+
6	34	+	neg.	+	+	+
7	38	+	neg.	+	+	+
8	40	+	neg.	+	+	+
9	48	+	neg.	+	neg.	+

Results of staining of renal biopsies with FITC-labelled anti-human immunoglobulins and complement components.

Circulating immune complexes

IgM and IgG, Clq-binding immune complexes were sought in the serum of six out of the nine patients by radioimmunoassay and none were found to be positive. Serum C3 levels measured in five patients during the acute phase were normal.

DISCUSSION

The pathogenesis of the HUS is unknown. Many different factors have been associated with the development of this syndrome (Kaplan, Thomson & de Chadarevian, 1976). However, the great variation in severity, incidence and geographical distribution emphasizes that the triad of haemolytic anaemia, thrombocytopenia and acute renal failure constitute the main features of a syndrome, and not a distinct clinico-pathological entity. This also explains the diversity of observations reported concerning coagulation and immunopathological studies in this syndrome.

Recent immunopathological studies have suggested the possible involvement of immune injury in the evolution of this syndrome (Cossio *et al.*, 1975; McCoy *et al.*, 1974; Cossio *et al.*, 1977). Glomerular deposits of immunoglobulin IgM and C3 have been reported in the acute phase of the disease (Cossio *et al.*, 1975; McCoy *et al.*, 1974). Complement components and immunoglobulin deposits have also been reported in chronic glomerulonephritis secondary to HUS (Cossio *et al.*, 1977). We have noted the presence of immunoglobulin and complement deposits after all clinical manifestations of renal disease had disappeared. Prior to these reports, many workers had failed to detect immune deposits. Though the

presence of such deposits might suggest the presence of immune complex injury, verification of such a mechanism requires definitive identification of a specific antigen in association with a specific immunoglobulin. Preliminary evidence for an antigen (Thomsen–Friedenreich antigen) has been reported (Klein *et al.*, 1977), thus lending support to the suggestion that the nephritic lesion observed is an immune complex induced injury. In addition, although previous efforts at antibody elution have been unsuccessful, thus suggesting non-specific trapping of macromolecules (Cossio *et al.*, 1975), the IgM that we observed was readily eluted. We failed to detect circulating immune complexes in the sera obtained from six of these patients during the acute phase. This may have been due to the cessation of complex formation after the initial renal damage had been induced by nephritogenic IgM-containing complexes. However, all these observations must be tempered by the fact that the syndrome is a manifestation associated with a heterogenous series of precipitating drugs and diseases.

Previous studies have presented evidence for complement activation in this syndrome (Kim, Miller & Michael, 1977). The deposition in glomeruli of complement components lends credence to the possibility of HUS being a reflection of immune complex injury (McCoy *et al.*, 1974; Cossio *et al.*, 1977). Cossio *et al.* (1977) thought that both alternative and classical pathway activation occurred as the components of both pathways, including C4, were localized in the glomeruli. C4 deposition is a common feature of nephritides associated with activation of the classical pathway of complement activation. In the majority of patients we studied with lupus nephritis, C4 deposition was observed consistently when both the classical and alternative pathways of complement were activated. The failure of intensive efforts to find C4 deposition in the glomeruli of the patients we studied is difficult to explain. However, the presence of IgM deposits associated with Clq in the early biopsies and properdin, C3 and Clq (in one case) in the later biopsies may be explained by possible *in vivo* activation of the Clq bypass activation pathway described *in vitro* by May & Frank (1973). In this pathway, aggregates of IgM will activate Clq through properdin, through properdin factor B, to C3 in the absence of C4. Such a method of complement activation has not been invoked in *in vivo* studies.

Our observations contribute further evidence of a possible immune mechanism in the pathogenesis of some forms of HUS. However, until definitive studies can determine a specific antigen as a causative factor, an immune mechanism as the sole pathogenesis cannot be definitively invoked. The possibility of the activation of the complement system operating as an initiator of coagulation and fibrinolysis (or vice versa) in this disease requires further study. Our observations suggest that complement activation by the Clq bypass activation pathway (May & Frank, 1973) may be present in this syndrome.

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