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Original article

Transplacental induction of membranous nephropathy in a neonate

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Abstract. We report a case of renal failure in a newborn infant due to membranous glomerulonephritis. The patient was anuric in the first 3 weeks of life, after which renal function recovered. The serum of the mother contained IgG antibodies which reacted with tubular brush borders and glomeruli of adult and fetal human kidneys. Reactivity with renal epithelium from human kidneys was detected. We suggest that a transplacental, passive Heymann nephritis-like mechanism was the pathogenesis of the neonate's symptoms, although the antigen(s) involved was shown not to be gp 330 or any of the renal antigens known to be involved in experimental nephropathies.

Key words: Kidney failure – Glomerulonephritis – Pathophysiology – Maternal-fetal exchange

Introduction

Renal failure is infrequent in the newborn and is associated most often with major perinatal complications or with congenital anomalies of the kidneys or urinary tract [1]. Glomerulopathy is a rare cause of renal failure in the newborn and is generally associated with congenital infections like syphilis and viral infections [1].

We report a case of transient renal failure due to membranous glomerulonephritis in a newborn. This disease appeared to have been induced by passively transferred maternal antibodies against kidney antigens. We investigated the maternal serum for reactivity against renal epithelium.

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Case report

Clinical history. The patient, a 2600 g male infant, was born after a first unremarkable pregnancy of 36 weeks to a 21-year-old mother. The parents were unrelated healthy individuals who had no family history of renal or autoimmune diseases. During pregnancy no drugs were used and there was no history of heavy metal intoxication. Maternal blood pressure, urinalysis and creatinine (65 μmol/l) were normal throughout the pregnancy as well as during a follow-up period of 2 years. Labour started spontaneously at home and only very little, if any, amniotic fluid was noticed. Because of failure to progress the patient was admitted to the local hospital, where vacuum extraction was performed. The child recovered spontaneously from a mild asphyxia with Apgar scores of 4 at 1 min, 7 at 5 min and 9 at 10 min. The boy was admitted to our hospital 2 days later because of anuria with a total urine production of 5 ml during the first 2 days after birth. The clinical condition had been unremarkable during the intervening period.

Physical examination revealed an active child with normal vital functions. There were no clinical signs of pulmonary hypoplasia and there were no congenital malformations. Two firm kidneys were palpable and the bladder was not enlarged. Ultrasound examination showed kidneys with increased echogenicity but of normal size (measuring 5.1 and 5.4 cm in length). There were no signs of obstructive uropathy. On the 3rd day after birth, arteriography and a kidney biopsy were performed, and, because of hyperkalaemia and fluid overload, peritoneal dialysis was started. Thrombosis of the renal arteries or veins was excluded on the basis of the arteriographic and histological findings and stable haematocrit and platelet counts. Immunological screening was performed only after the biopsy results became available. There were no clinical signs of infection until day 12, when a *Staphylococcus aureus* peritonitis was treated with gentamicin and cefamandole. The gentamicin was administered on the basis of serum trough levels. All clinical and circulatory parameters were stable in this period.

Diuresis amounted to a total of only 75 ml until day 25. After this it gradually increased without signs of polyuria. The peritoneal dialysis catheter was removed 10 days later and plasma creatinine decreased to 60 μmol/l within 2 months after birth. Follow-up during 2 years revealed no clinical problems, no haematuria or proteinuria and a stable creatinine clearance of 75 ml/min per 1.73 m^2 at 1 year.

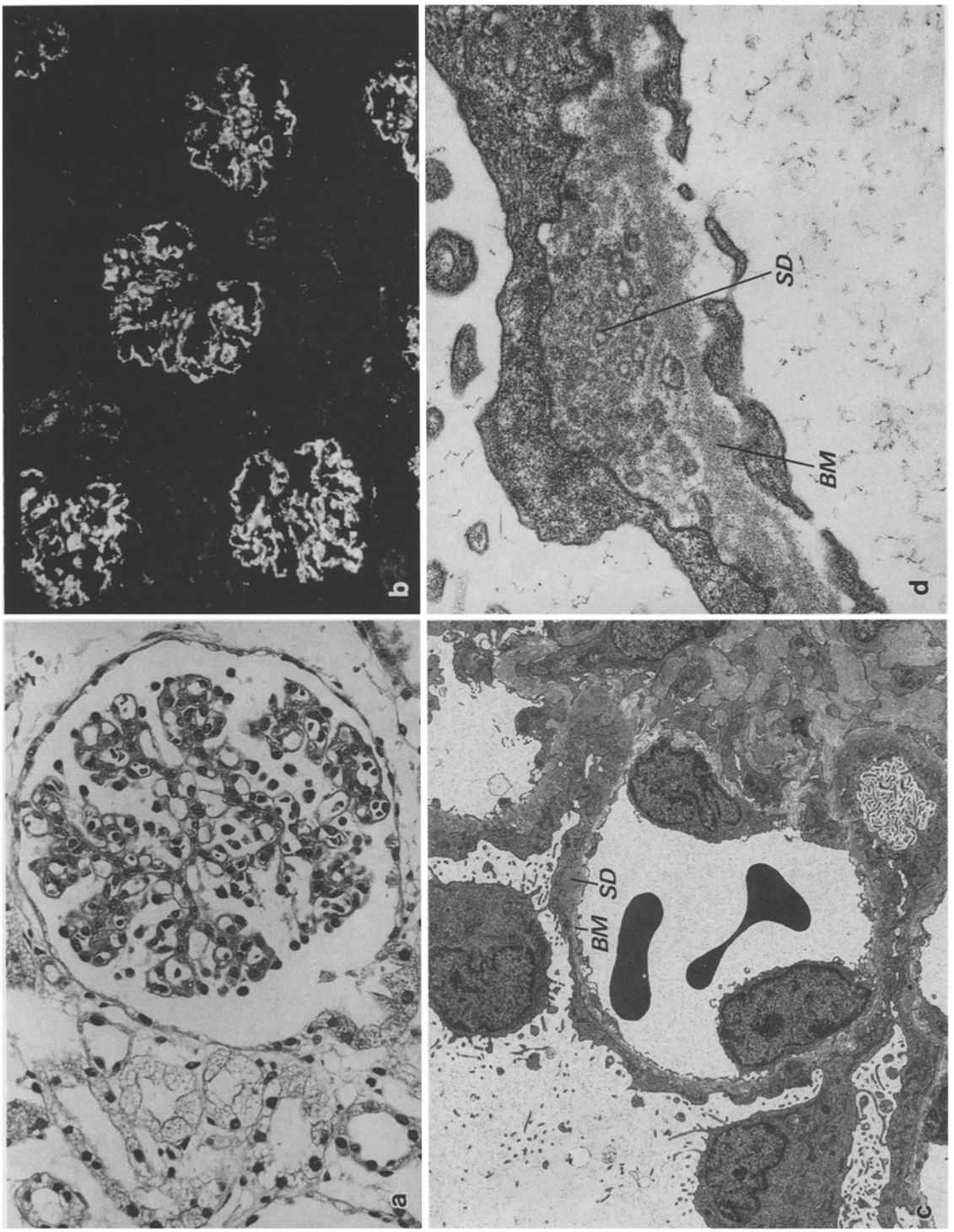

Fig. 1a–d. Renal biopsy of the patient. **a** A representative glomerulus is shown with accentuated lobular architecture. Haematoxylin azofloxin, × 380. **b** Granular IgG deposits along the basement membranes of all glomeruli, × 350. **c** An ultrastructural picture of a capillary loop with sub-epithelial deposits (*SD*) along the basement membrane (*BM*), × 1500; higher magnification (**d**) demonstrates circular structures in these deposits, × 18,000

Laboratory investigations. The child's renal parameters deteriorated progressively during the first days after birth. At the start of dialysis serum sodium was 125 mmol/l potassium 6.5 mmol/l creatinine 921 μmol/l urea 21 mmol/l and the plasma pH 7.40. There were no signs indicative of increased haemolysis. The Coombs test was negative and there was no abrupt fall in haematocrit or platelet count; the initial platelet count was 180,000/mm^3 and the initial haematocrit was 63%.

Many bacterial and viral infections could be excluded by serological studies of mother and child. These included syphilis, toxoplasmosis, streptococcal infection and infections by herpes simplex, Epstein-Barr (EB) rubella, cytomegalo- and hepatitis B viruses. There was a slightly elevated maternal antibody level against measles virus using a complement fixation technique (1:64). There was no neonatal jaundice and plasma levels of liver enzymes were always normal. Immunological

tests of the patient and his mother were performed in the 3rd week after birth. Rheumatoid factors and antibodies against nuclear factors were not elevated. Antibodies against lymphocytes and several tissue antigens including parietal cells, adrenal cortex, mitochondria, skin and thyroid tissue antigens could not be detected by indirect immunofluorescence (IF). Antibodies against renal antigens, as characterised later, were detected in the mother but not in the child. Circulating immune complexes could not be detected by C1q binding assay in the plasma of mother or child. The mother had normal complement C3, C4, C1q and CH50 and the child had complement values (C1q, 0.11 g/l; C3, 0.7 g/l; C4, 0.16 g/l; CH50, 49%) within the normal range given by Jolliff et al. [2].

Kidney biopsy. The kidney biopsy specimen contained about 50 glomeruli and was studied by light, immunofluorescent and electron microscopy. All glomeruli (Fig. 1) showed the same slightly immature picture: pronounced visceral epithelial cells were arranged in rosettes around the capillary loops. In all glomeruli the lobular architecture was accentuated by a slight increase in mesangial cells and matrix. The capillary lumina were expanded and contained erythrocytes and some polymorphonuclear leucocytes. Silver stain methods revealed only minimal changes of the basement membranes. Focally some irregularities on the outer surfaces of these membranes were seen without the typical characteristics of spikes. The renal interstitium was nearly normal and the vessels showed only slight swelling of the endothelial cells. The convoluted renal tubules were covered by cylindrical epithelial cells with pale, vacuolated foamy cytoplasm showing hyaline droplets. Some tubules were somewhat dilated. The epithelial covering of these tubules was cuboidal or flattened.

IF studies revealed extensive deposits in all glomeruli of IgG, C3, C9 and to a lesser degree of IgM, C1q and properdin in a clear fine granular pattern along the basement membranes. No deposits were present in vascular walls. The epithelium of the convoluted proximal tubules had extensive granular fluorescent staining for albumin and to a lesser degree for IgG and C3.

Ultrastructural studies revealed fusion of the foot processes of the visceral epithelial cells of all glomeruli. Extensive electron-dense deposits containing many circular virus-like particles were detected in an epimembranous distribution along the glomerular basement membranes (GBM). The endothelial cells showed only slight changes. The tubular epithelial cells showed extensive cytoplasmic vacuolization. Since the maternal serum contained antibodies to measles virus, we stained the patient's kidney for this virus. No binding of anti-measles monoclonal antibodies could be detected by IF.

Immunochemical characterization of maternal antibodies. Brush border (BB) membranes were prepared by hypotonic lysis, calcium precipitation and centrifugation according to the methods of Malathi et al. [3] from saline-perfused normal human cadaveric kidneys (a generous gift from Dr. G. Persijn, Eurotransplant, Leiden). Human renal tubular epithelium glycoprotein (hRTE-gp) was prepared by solubilization of these BB membranes in 1% sodium deoxycholate, gel filtration on Sephacryl S-300 and affinity chromatography on Lentil lectin-Sepharose B as previously described for rat [4] and for mouse kidneys [5]. The human analogue for gp 330 was purified by HPLC gel filtration of hRTE-gp on a TSK Ultrapac 300 SW column (Pharmacia-LKB, Uppsala Sweden). Gp 330 was detected in column fractions by ELISA using eluted rat antibodies from Heymann kidneys [6]. SDS-PAGE analysis under reducing conditions [7] revealed one major band with an apparent molecular weight of 440 kDa and one minor band at 110 kDa, as shown by others [8, 9]. Affinity-purified rat gp 330 and human fibrinogen (350 kDa) were run separately as molecular weight markers. The procedures for immunofluorescence on kidney section and the preparation of FITC-conjugates have been described previously [10]. For absorption of maternal serum, aliquots of 100 µl were incubated with 1 mg hRTE-gp in 100 µl phosphate-buffered saline and incubated for 30 min at 4° C on a gyrotory shaker and for 30 min on ice. Human glomerular basement membrane (hGBM) was prepared from glomeruli which were isolated from a saline-perfused human cadaveric kidney by sieving and centrifugation. Collagenase-digested GBM was prepared as described previously for rat kidneys [11]. Mouse Engelbreth-Holm-Swarm (EHS) laminin, human collagen type IV and human fibronectin were purchased from Sigma, St. Louis, Mo., USA. Affinity-purified human dipeptidyl aminopeptidase IV (DDP IV) was a generous gift from Drs. Ronco and Verroust, Hôpital Tenon, Paris.

Results

IF studies using serum of the mother on frozen sections of a panel of ten normal adult human kidneys showed heavy staining on the BB of the proximal tubules. This was in contrast with results obtained with serum from healthy controls and from pregnant women. Diffuse granular staining was also observed in the glomeruli (Fig. 2a). This glomerular staining was equally strong or slightly stronger when serum was applied to frozen sections of human fetal kidneys (15–18 weeks old) (Fig. 3). Staining of the BB, however, was stronger on adult than on fetal kidneys. Maternal serum produced staining of equal intensity on all tested human kidneys, indicating that antibodies were not directed against allospecific antigens. Serum obtained from the baby at 3 weeks of age was negative by IF on adult kidneys.

In order to investigate the specificity of these antibodies further, several antigens were purified from human cadaveric kidneys as described above. Absorption of maternal plasma with hRTE-gp resulted in a reduced BB staining of adult human kidneys (Fig. 2b). However not all anti-BB activity could be removed, indicating that the mother's antibodies were not exclusively directed against RTE-gp.

Testing, however, of the maternal antibodies against purified human gp 330 by ELISA did not result in a significant signal, which excluded reactivity with this glycoprotein. The sera were also negative when tested by ELISA against affinity-purified human DPP IV, or against collagenase-digested human hGBM, collagen IV, fibronectin and mouse EHS-laminin.

Maternal plasma, obtained 2 years later, still showed strong BB staining on normal human kidneys by IF.

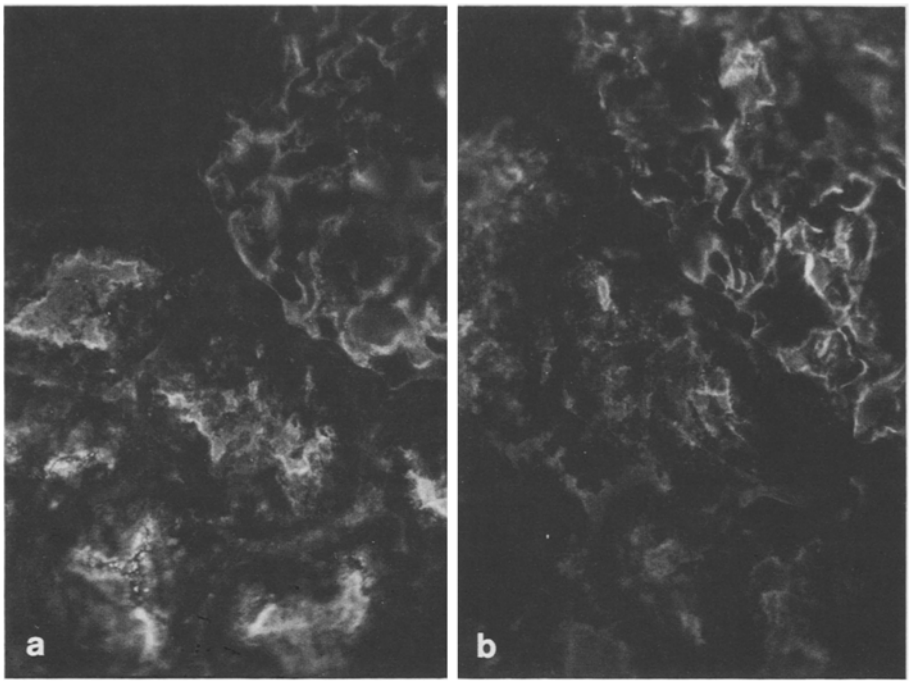

Fig. 2. Typical indirect immunofluorescence pattern of the mother's serum on an adult kidney section: **a** before and **b** after absorption with purified human renal tubular epithelium glycoprotein. Note the partial disappearance of brush border staining, while the glomerular staining remained unaffected. × 300

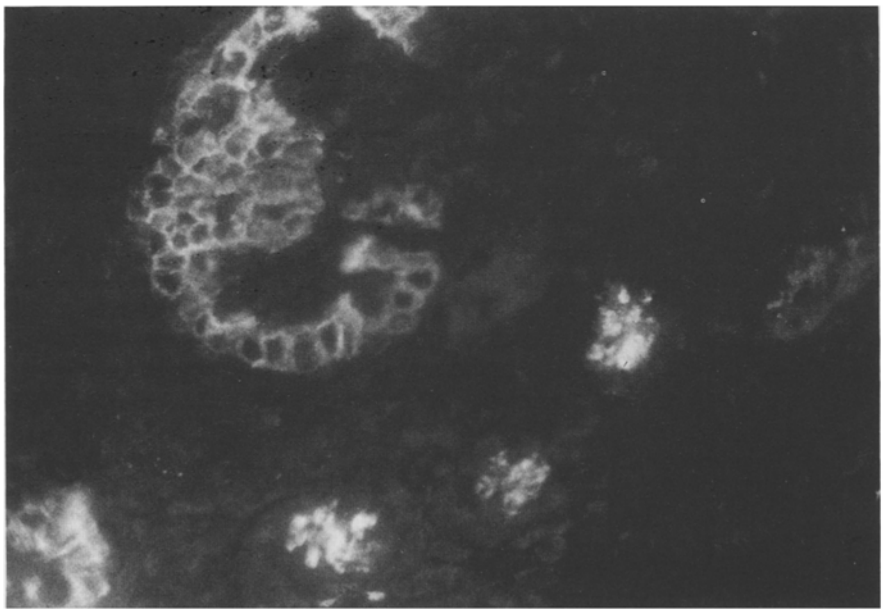

Fig. 3. Indirect immunofluorescence of the mother's serum on a fetal kidney (15 weeks old). Note the relatively strong glomerular staining × 480

Discussion

Although membranous immune complex glomerulonephritis in a newborn without congenital infection has not yet been reported, the histological findings strongly suggest that this was the aetiology of the clinical symptoms of our patient. Alternative causes of neonatal renal failure were excluded. The circular particles observed in the subepithelial deposits of the renal biopsy might indicate a viral infection. However, these particles are well-recognised features in membranous glomerulonephritis and are assumed generally to be non-viral degradation products [12]. Unequivocal demonstration of virus has been exceptional and usually restricted to hepatitis B antigen [13] or EB virus [14]. We assume that the particles demonstrated were non-viral products since many viral infections, including EB and hepatitis B viruses, were serologically excluded.

We were impressed by the prolonged anuria which is not generally a feature of this type of glomerulonephritis when it presents later in life. Three factors might have enhanced renal failure in this patient. First, the susceptibility to renal injury might be increased in the neonate as a result of the unique haemodynamic conditions of the neonatal kidney. Neonates have a low glomerular filtration rate as a consequence of a low blood pressure, a high renal vascular resistance, a low renal blood flow and a low ultrafiltration coefficient [15]. Secondly the extensive deposits seen in the biopsy material might be related to the higher affinity of the maternal antibodies for fetal renal antigens than for adult renal antigens. Finally, it can never be fully excluded that an undetected circulatory imbalance in the perinatal period aggrevated renal failure.

Although not yet reported as a cause of glomerulonephritis, we propose that this case was induced by transplacentally transported maternal IgG antibodies to unknown renal antigens. The abundant presence of IgG in the child's glomeruli, the presence of circulating anti-renal IgG antibodies in the mother but not in the child at 3 weeks after birth and the complete recovery of renal function shortly after birth support this idea. It is well known that IgG is actively transported across the placental membrane [16]. Also several maternal diseases that are immunologically mediated, such as Graves disease, systemic lupus erythematosus and myasthenia gravis, can be manifested temporarily by infants and maternal C3 nephritic factor can appear transiently in the sera of newborns [17, 18]. Jordan et al. [19] described the transplacental induction of transient distal tubular dysfunction by IgG in the newborn of a mother with Sjögren's syndrome [19]. Glomerulonephritis or acute renal failure were not, however, present in that case or in the reported cases of neonatal systemic lupus erythematosus.

The absence of clinical signs of glomerulonephritis in the mother might be related to a difference in antigen expression by adult and fetal kidneys. Allospecificity is a less likely explanation for the absence of maternal symptoms, since the maternal antibodies reacted with all kidneys of the panel in the IF study. Moreover allospecificity has not yet been reported for this category of anti-renal antibodies.

This case has pathohistological features in common with the model of passive Heymann nephritis. In this model a heterologous immune complex glomerulonephritis can be induced in various strains of rats by a single injection of heterologous antibody directed against antigens present in the BB of the proximal tubules of the rat kidney [20].

Only a few renal glomerular antigens have been described that can serve as fixed antigens for in situ subepithelial immune complex formation in the glomerulus by circulating antibodies. Most of these antigens have been characterized in rodents and lagomorphs. In the rat gp 330 [21], gp 90 [22] and gp 108 [23] have been described, but the latter two have both been identified as DPP IV [24, 25]. In the rabbit an epithelial foot process antigen [26] has been shown to be the target for membranous glomerulopathy. In spite of elaborate investigations no intrinsic human glomerular epithelial antigen has been demonstrated in cases of membranous glomerulopathy [27, 28]. However, a few reports have suggested this involvement [29, 30].

We conclude that this patient had a membranous glomerulonephritis which was most likely induced by transplacental transfer of maternal antibodies against human renal epithelium. The maternal antibodies were shown not to be directed against any of the nephritogenic proteins known to be involved in experimentally induced nephropathies in rodents and remain unidentified as yet. We cannot yet conclude whether this specificity has any nephritogenic significance for the human fetal kidney.

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