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Renal pathology of Waldenström's macroglobulinaemia with monoclonal antiglomerular antibodies and nephrotic syndrome

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SUMMARY

Severe nephrotic syndrome developed suddenly in a 67-year-old man. IgM M-component and bone marrow findings indicated a diagnosis of Waldenström's macroglobulinaemia. High-titre IgM glomerular autoantibodies were found to reside mainly in the M-component. Immunofluorescent (IF) studies on serial kidney biopsies showed extensive IgM deposits that disappeared after treatment. Light microscopy of kidney biopsy appeared only slightly altered, but combined with electron microscopy could demonstrate changes that correlated well with IF findings. The latest biopsy showed interstitial infiltration in the kidney of atypical lympho-histiocytic cells. Morphological and immunological examination indicated that pathogenetic events started with minimal-change glomerulonephritis, causing release of glomerular antigen, that finally triggered a monoclonal IgM response and lymphoproliferative reaction. Intermittent treatment with chlorambucil and corticoids completely abolished the nephrotic syndrome and at the same time the changes in renal morphology largely disappeared.

INTRODUCTION

The incidence of renal complication in Waldenström's macroglobulinaemia (WM) is low compared to the incidence in myeloma patients. This is probably related to the rare occurrence of Bence–Jones proteinuria in WM (MacKenzie & Fudenberg, 1970; Waldenström, 1965).

Sixteen patients with WM were studied in 1970 with respect to renal function, proteinuria and renal histology (Morel-Maroger *et al.*, 1970). Immunofluorescence studies in six patients showed striking voluminous deposits on the endothelial aspect of the glomerular basement membrane, consisting exclusively of IgM. The deposits were thought to result from passive deposition due to the physical-chemical properties of the IgM molecule including high concentration, hyperviscosity and possibly cryoprecipitability. No definite correlation was established between the presence of IgM deposits, proteinuria and renal failure. In two additional patients studied by immunofluorescence, including one with nephrotic syndrome, thready fixation of anti-IgM in glomerular arteriolae and peritubular capillaries was found. These were interpreted as deposits of circulating IgM, but no explanation of the nephrotic syndrome was found. Light microscopy showed no abnormalities in the renal biopsy specimen.

More recently a case has been reported in which WM was associated with nephrotic syndrome, and where renal disease was thought to be immunologically mediated by immune complex

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deposition. This assumption was based on low serum complement levels and granular deposits of IgM, IgG and C3 along the glomerular basement membrane (Martelo *et al.*, 1975).

We report here a case of WM with nephrotic syndrome where the patient's monoclonal IgM showed antibody activity against glomerular antigens and where IgM deposits were demonstrated in glomeruli and interstitial capillaries. The renal involvement was followed by repeated biopsies, examined both light and electron microscopically. We were also able to confirm the excellent effect of intermittent chlorambucil-prednisone treatment on the nephrotic syndrome.

CASE HISTORY

The patient was a white man aged 67 years. He was a farmer and had been well previously. His symptoms appeared gradually in June 1976 with fever, arthralgia, shortness of breath and marked oedema. On examination he was found to have gross oedema of both legs and the abdominal wall. Chest X-ray revealed fluid in the pleural cavities. The erythrocyte sedimentation rate was 116 mm/hr, increasing to 159 mm/hr. There was albuminuria of 5·2 g/l, increasing to a maximum of 8·8 g/l 2 months later. Urine electrophoresis showed a selective type of glomerular proteinuria consisting mainly of albumin and a transferrin band (Fig. 1). Serum albumin was 20 g/l on admission and decreased to 15 g/l. Microscopy of urine revealed numerous casts but only a few red blood cells and polymorphonuclear leucocytes per field. The haemoglobin was 90 g/l, and the white blood cell count (including differential count), thrombocyte concentration and red cell morphology were within normal limits. Serum creatinine was within normal limits. The serum cholesterol concentration was 15·8 mmol/l while serum triglycerides were 2·4 mmol/l (normal ranges 4·2–8·6 mmol/l and 0·4–2·2 mmol/l respectively). Serum electrophoresis revealed an M-component in the gamma region (Fig. 1), and immunoelectrophoresis classified it as an IgM M-component bearing kappa light chains. Ig-quantitations using electroimmune diffusion according to Laurell (1966) showed IgG 5·0 g/l, IgA 2·1 g/l, and IgM 19·4 g/l, subsequently increasing to a maximum of 32 g/l 3 months later. (Normal Ig ranges in our laboratory are: IgG 7·0–14·0 g/l; IgA 1·2–3·5 g/l; IgM 0·5–1·8 g/l.) Immunochemical measurements of complement components (C3, C4, C1q, C1s) were all within normal limits. Rheumatoid factor tests (Latex [Hyland Laboratories] and Rose–Waaler) were negative, as was also Coombs' direct antiglobulin test. No cryoglobulin could be demonstrated in the serum. Bone marrow examination showed an increase in atypical lymphoid cells with the appearance of lymphoblasts. An increase in plasma cells and mast cells was also noted. The diagnosis of Waldenström's macroglobulinaemia was thus confirmed.

Morphology

Percutaneous needle biopsies were performed on three occasions: initially for renal diagnosis, after

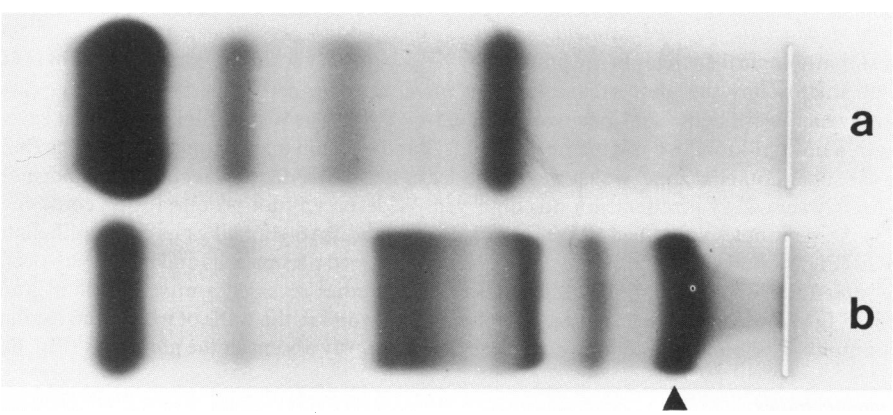


Fig. 1. Agarose gel electrophoresis of (a) urine and (b) plasma, obtained before treatment was started. Arrow indicates M-component.

5 days of prednisone-chlorambucil treatment and 1·5 years later. Tissue portions were processed for light and electron microscopy by fixation in 5% glutaraldehyde and 1% OsO_4, dehydrated and embedded in Epon–Araldite. Semithin sections were stained by toluidine blue and by periodic acid–Schiff (PAS) after dissolving the epoxy resins with sodium-methoxide. Ultra-thin sections were stained by uranyl acetate and lead citrate and observed in a Philips EM 300 electron microscope. Specimens for light microscopy also included examination of 1-μm plastic (Sorvall, JB-4) and 7-μm paraffin sections stained with PAS and alcian blue. Trichrome stains were also used for demonstration of the glomerular basement membrane and the methyl-green method for demonstration of plasma cells. For detection of amyloid the alkaline Congo red method was used with examination in polarized light.

Portions of the biopsies were snap-frozen in liquid nitrogen for immunofluorescence studies. Human IgG, IgM, IgA, β1c and fibrinogen were stained by applying appropriate FITC-conjugated antisera (Wellcome Ltd) to unfixed 3-μm cryostat sections (Coons & Kaplan, 1950). Microscopy was carried out with a Zeiss incident-light fluorescence microscope.

Serological studies

Unfixed 5-μm sections of mouse kidney, stomach, lung, liver and spleen were used as substrate to detect non-species-specific autoantibodies against tissue constituents by the indirect immunofluorescence technique. The autoantibody character of anti-tissue antibodies detected was also proved on human kidney sections. The sera were tested at a 1/10 dilution for autoantibodies of IgG, IgM and IgA classes by applying appropriate FITC-conjugated antisera. FITC-conjugated kappa and lambda antisera (DAKO Immunoglobulins A/S, Copenhagen, Denmark) were used to study whether detected autoantibodies were bearing preferentially kappa or lambda light chains. The fluorescence titre was established with increasing dilutions until an end-point was reached. Glomerular basement membrane (GBM) and rabbit anti-GBM serum were prepared according to Marquardt, Wilson & Dixon (1973) for blocking experiments.

In vivo fixation studies

Fixation of the antiglomerular autoantibodies *in vivo* was tested by injection of 0·5 ml undiluted serum from the patient into the tail vein of mice. Normal human sera and sera from WM patients without renal complications were used as controls. Tests for proteinuria and haematuria were done after 18 hr with Labstix (Miles Laboratories Ltd). The mice were then killed, and renal tissue snap-frozen for immunofluorescence studies. Deposits of human IgM, IgG and IgA were stained by applying appropriate FITC-conjugated antiserum.

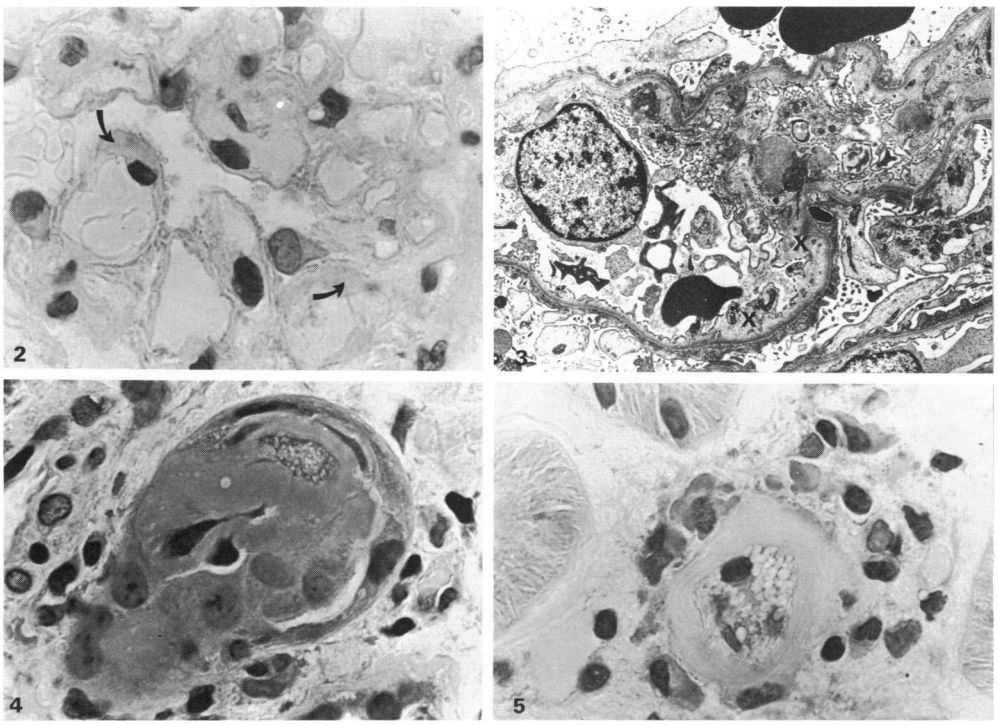
RESULTS

Morphological studies before treatment

The histopathological changes in the first kidney biopsy, mostly from the cortex, were not striking. On light microscopy the glomerular basement membrane seemed to be thickened by scattered deposits which were dense and positively stained by PAS. The alcian blue–PAS stain revealed, however, a normal-looking basal membrane (Fig. 2) and electron microscopy localized the thickening to the subendothelial zone, which was expanded. The lamina densa was completely normal. No endo- or extracapillary proliferation was observed. Widespread foot process fusion could be seen (Fig. 3). Some tubules contained dense, homogeneous and PAS-stained casts with epithelial cells included (Fig. 4). Staining for amyloid was negative. Scattered plasma cells and histiocytes occurred in the interstitial tissue (Fig. 5). Immunofluorescence studies revealed a mixed fibrillar-granular deposit of IgM along the glomerular basement membrane and in the walls of interstitial capillaries. Other immunoglobulins and complement were conspicuously absent in the glomeruli (Fig. 6a).

Serological studies

The patient's serum showed a high titre (1/6,400) of IgM autoantibodies against mouse as well as human glomeruli (Fig. 6b). There was no staining of other tissue components tested such as smooth



Figs. 2–5. *First kidney biopsy*

Fig. 2. Part of the glomerular capillary tuft, showing dense, PAS-positively stained subendothelial deposits (*arrows*). Note the well preserved and thin basement membrane. The capillary lumina are free from thrombi (or so-called 'coagula'). (Alcian blue–PAS, × 650.)

Fig. 3. Electron micrograph of a glomerular capillary loop. Note the wide subendothelial zone (X) over a well preserved lamina densa of the basement membrane. The foot process fusion is extensive and resembles the appearance seen in glomerulonephritis of the minimal-change type. (× 3,000.)

Fig. 4. Tubule containing dense cast which includes groups of epithelial cells resembling the syncytia, seen in multiple myeloma. (Alcian blue–PAS, × 650.)

Fig. 5. Atrophic tubule, showing strikingly thickened basement membrane, closely surrounded by plasma cells and histiocytes. (Alcian blue–PAS, × 650.)

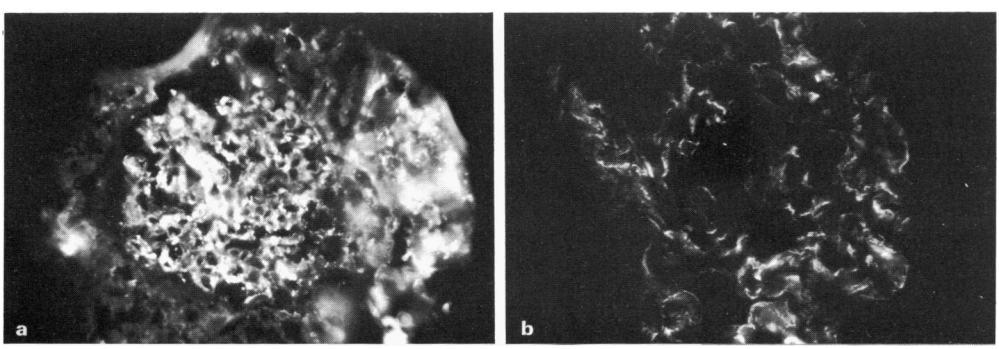


Fig. 6. (a) Immunofluorescent studies of the first kidney biopsy. Heavy fibrillar-lumpy deposits of IgM along GBM and in mesangial area. Also prominent speckled interstitial IgM staining, mainly localized to tubular basement membrane. No staining was noted when antisera against IgG, IgA and C3 were used. (b) Indirect immunofluorescence (anti-IgM) with human kidney as substrate showing fine granulae scattered diffusely in glomeruli. No staining was noted with antisera against IgG or IgA.

muscle or tubular basement membrane. The antiglomerular activity could not be blocked by anti-GBM serum. There was no fixation of the patient's IgG or IgA to the substrates used. However, it could be shown by applying FITC-conjugated light chain antisera that the antiglomerular activity was exclusively dependent on IgM antibodies bearing kappa light chains.

In vivo fixation test

Injection into mice of the patient's serum, normal human serum, and sera of patients with WM but without renal complications did not give proteinuria or haematuria. Immunofluorescence examination of the kidney tissue showed scattered IgM deposits in the glomeruli of mice given the patient's serum and sera from patients with WM but without complications. These deposits were regarded as non-specific as there was no difference in effect between WM serum with or without antiglomerular antibodies when increasing dilutions were used (Kijlstra *et al.*, 1978).

Analysis of serum and proteins

Plasma viscometry was done repeatedly using a Wells–Brookfield cone-plate viscometer. Initially a value of 2·05 nmol/m^2 was obtained, indicating elevated viscosity (normal range 1·20–1·60 nmol/m^2). Subsequently plasma viscosity increased to 2·30 nmol/m^2 despite plasmapheresis on two occasions using a Haemonetics blood cell separator (total of 1,260 ml). After institution of chemotherapy plasma viscosity returned to normal, and plasma IgM levels decreased from 20 to 15·4 g/l but later tended to increase slightly.

Treatment and progress

The result of treatment is illustrated in Fig. 7. No improvement was seen after limited plasmapheresis, but there was a dramatic response to intermittent prednisone-chlorambucil treatment

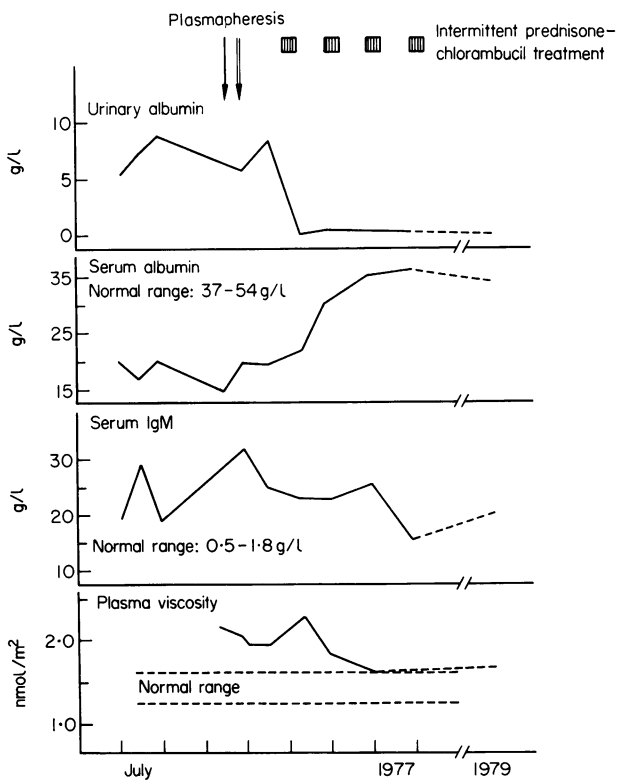
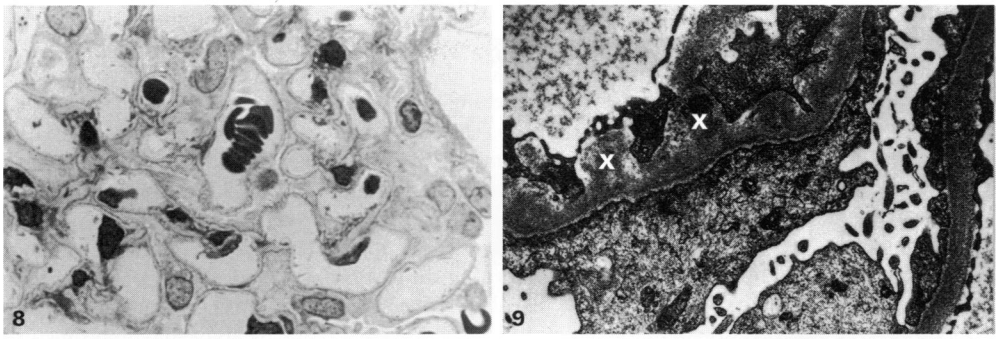
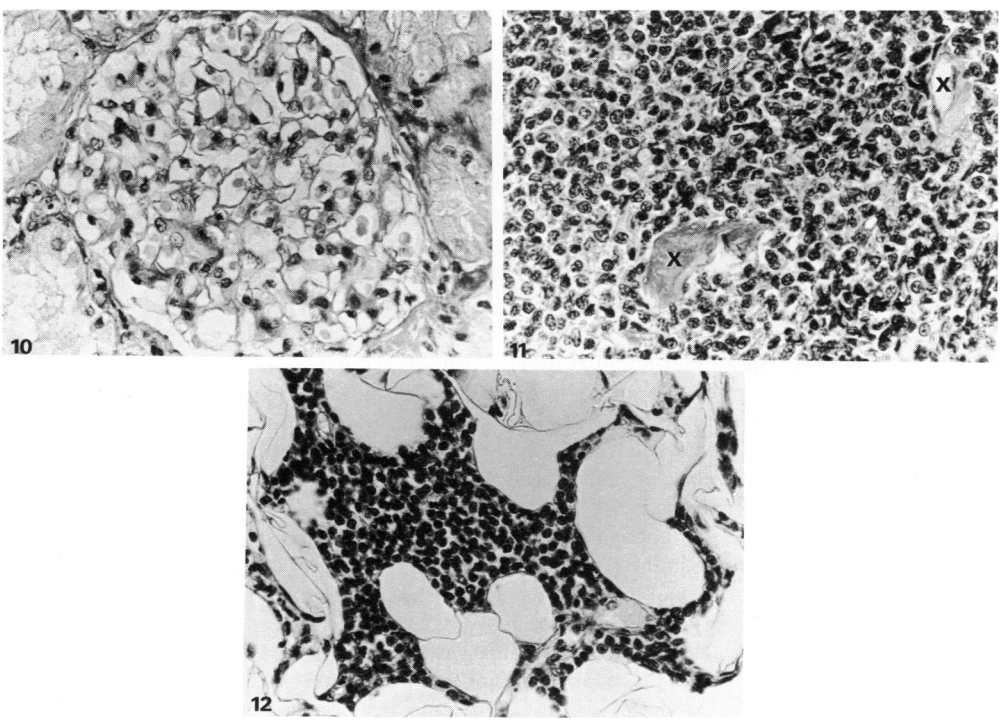


Fig. 7. Reversal of nephrotic syndrome, normalization of S-viscosity and reduction in S-IgM levels in relation to introduction of intermittent prednisone-chlorambucil treatment.



Figs 8 and 9. *Second kidney biopsy (after 5 days' treatment)*

Fig. 8. Glomerular capillary loops of apparently normal appearance. No detectable subendothelial deposits. No cellular proliferation and the basement membrane seems to be well preserved and thin. (Toluidine blue × 650.)
Fig. 9. Electron micrograph, showing irregular thickening focally of glomerular basement membrane by subendothelial deposits (X) facing fused epithelial foot processes. In other areas (to the right) the epithelial pedicles are seen to be normal. (× 10,300.)



Figs. 10–12. *Third kidney biopsy (1·5 years after the first one)*

Fig. 10. Glomerulus of normal appearance. There are no signs of deposits or cellular proliferation. (Trichrome stain [Ladewig], × 250.)
Fig. 11. Dense medullary interstitial cellular infiltrates made up by atypical lymphocytes and histiocytes with large nucleoli. The tubules (X) are invaded and destroyed by the infiltrates. Note absence of fibrosis separating this condition from chronic interstitial nephritis. (Trichrome stain [Ladewig], × 310.)
Fig. 12. Atypical cell infiltrate in pelvic fat tissue. The same cellular composition is seen as in the medullary interstitium (Fig. 10). (van Gieson, × 250.)

(chlorambucil 8 mg daily for 5 days and prednisone 125 mg daily for 3 days at monthly intervals). A kidney biopsy 2 months after the first one (after 5 days of medical treatment) has shown apparently normal glomeruli without any light microscopically detectable deposits (Fig. 8). Electron microscopy revealed, however, a localized, severe thickening of the GBM due to subendothelial deposits. Areas of foot process fusion also remained (Fig. 9). The immunofluorescence pattern was essentially identical to that of the first biopsy. At present, 32 months after the first appearance of symptoms, the patient is symptom-free on a normal salt diet and without diuretics. The proteinuria has completely disappeared, and the serum albumin level has returned to normal. The nephrotic syndrome has thus been completely reversed. The patient is receiving regular, intermittent treatment with chlorambucil-prednisone. In a third renal biopsy 1·5 years after the first the glomeruli still appeared normal (Fig. 10) and no immune deposits could be revealed by the immunofluorescence technique. There was, however, a supervention of dense cell infiltration in the renal tissue by atypical lympho-histiocytic cells, mostly localized to the medullar interstitium (Fig. 11) but also invading tubuli, vessels and pelvic fat (Fig. 12). Very few of them showed staining characteristics for mature plasma cells. Serum IgM levels now remain at 15–20 g/l and peripheral blood values are normal at the time of writing.

DISCUSSION

The histological features in our case are remarkable. The initial lesions were characterized by glomerular membrane thickening, epithelial foot process fusion and tubular casts, which is in agreement with results obtained by other investigators (Heptinstall, 1974; Larcan, Rauber & Streiff, 1962; Morel-Marager *et al.*, 1970; Morel-Maroger & Verroust, 1974). In the present study we were able to localize the glomerular basement membrane lesion to subendothelial deposits which disappeared after treatment. This event paralleled the regression of clinical symptoms. The absence of glomerular intracapillary thrombi ('coagula') and the presence of subendothelial deposits suggest a different mechanism for IgM deposition than hyperviscosity in this case. The repeated morphological follow-up disclosed, on the other hand, progression of the disease with lymphohistiocytic cell infiltration. These were found to be localized almost completely to the medulla, in contrast to the findings by others (Hamburger, 1969; Morel-Maroger *et al.*, 1970) who saw them mainly in the cortex. The cellular composition and the lack of fibrosis make these infiltrates very characteristic and they may thus be regarded as a valuable diagnostic aid. It may also be maintained that a kidney biopsy, which lacks medullary tissue, does not seem to be satisfactory in recognizing the renal involvement in WM. The fibrillar-granular IgM deposits observed in the kidney of our patient differ from the typical granular deposits seen in immune complex-mediated glomerulonephritis (Dixon, Feldman & Vazquez, 1961), the linear deposits seen in anti-GBM glomerulonephritis (Duncan *et al.*, 1965) and the type of IgM deposit usually seen in WM kidneys (Morel-Maroger *et al.*, 1970). The presence and disappearance of IgM deposits correlated well with the subendothelial deposits seen in light and electron microscopy.

The very high titre (1/6,400) of IgM autoantibodies directed against kidney glomeruli would suggest that the IgM deposits in the patient's kidneys were a result of a specific antigen–antibody reaction. This seems, however, not to be the case as there was no increased fixation of IgM from our patient to mouse glomeruli *in vivo* compared to WM sera lacking specific glomerular autoantibodies. This may indicate that the glomerular antigen is not available *in vivo*, possibly owing to intracellular localization. The lack of inhibition using anti-GBM antiserum in blocking experiments indicates that the IgM antibody in this case has a different specificity than the anti-GBM antiserum. The monoclonal IgM nature of the antiglomerular antibodies was indicated by IF experiments where IgM localized to glomerular structures had only kappa-type light chains. Furthermore, the absence of C3 deposits as well as the morphological findings speak against a glomerular injury based on an antigen–antibody reaction.

The absence of IgM deposition in the latest kidney biopsy, at a time when the patient still had high serum levels of monoclonal IgM, indicates that a prerequisite for IgM deposition is a change in the glomerular basement membrane; when the GBM has been repaired as a result of the combined

treatment, IgM deposits will no longer take place. That changes in the structure and/or function of the GBM predisposes to deposition of various serum proteins might thus be suggested.

The minimally altered morphology on light microscopy but the extensive epithelial foot process fusion on electron microscopy and the disappearance of the selective glomerular proteinuria with the chlorambucil-corticoid therapy indicate that our patient initially suffered from a non-immunological glomerulonephritis of the minimal-change type. This type of glomerular injury may well result in a release of glomerular cell antigens into the circulation. In predisposed individuals prolonged exposure to the glomerular antigen might result in a finally monoclonal IgM response. The large quantities of IgM could then cause further kidney damage as a result of physico-chemical deposition in altered glomeruli due to hyperviscosity and poor clearing capacity of mesangial cells. The suggested pathogenetic mechanisms are depicted in Fig. 13.

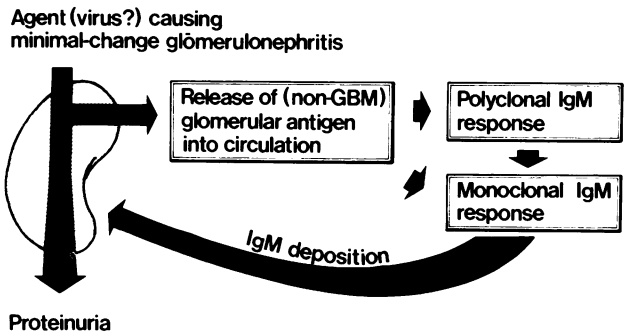


Fig. 13. Hypothetical pathogenetic mechanisms leading to kidney damage. See text for comment.

It is likely that the dramatic improvement with disappearance of the nephrotic syndrome depends mainly on the effect of corticoids on the primary renal disease (minimal-change glomerulonephritis) (Idelson *et al.*, 1977), since the improvement occurred and persisted despite high serum levels of monoclonal IgM. The combined chlorambucil-corticoid therapy, however, would affect not only the basic glomerular disease but also has a lympholytic effect, causing a reduction in IgM synthesis. That this immunosuppressive effect is partly responsible for the improvement is indicated by the close relation in time between clinical improvement and normalization of serum viscosity and reduction in serum IgM level. The glomerular disease seems to have been reversed, but the lympho-histiocytic cell infiltration seen in the latest kidney biopsy may indicate that the kidneys are now involved in a lymphoproliferative process.

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