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Case Report

Macroglobulinemia with Abdominal Symptoms Caused by Intestinal Extracellular Macroglobulin

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Summary. A 54-year-old woman had a marked splenomegaly, diarrhoea and abdominal cramps. Her serum contained monoclonal IgM with lambda light chains, and lambda light chains were also excreted in the urine. Bone marrow and spleen punctures failed to reveal the classic morphological changes associated with Waldenström's macroglobulinemia. A peroral jejunal biopsy disclosed homogenous extracellular material consisting of IgM lambda immunoglobulin associated with kappa light chains and lipids. Treatment with chlorambucil and prednisolone had no effect. IgM, lambda and kappa light chains were demonstrable in a large number of the plasma cells and plasmacytoid cells in post-mortem specimens of bone marrow, spleen and some lymph nodes by the peroxidase-anti-peroxidase techniques. The causes of the "discrepancy" between the serum findings and tissue findings are discussed.

Key words: Extracellular intestinal macroglobulin – Intracytoplasmic immunoglobulin – Malabsorption – Macroglobulinemia.

Introduction

A number of lymphoproliferative diseases, including plasma cell tumours in the broad sense of the concept, involve infiltration of the intestinal mucosa by neoplastic cells to an extent sufficient to cause intestinal symptoms and a malabsorption syndrome (cited by Pruzanski et al. 1973). Accumulation of macroglobulin in the intestinal mucosa in macroglobulinemia is extremely rare (Pruzanski et al. 1973), and Baker et al. (1971) were the first to detect the intestinal lesion by peroral biopsy. To our knowledge, the study of Pruzanski et al. (1973) is the only one in which the macroglobulin accumulating in the intestinal mucosa has been identified immunohistochemically.

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We describe a patient who presented with symptoms including diarrhoea and abdominal cramps and for whom a diagnosis of macroglobulinemia was established on the basis of immunohistochemical analysis of a peroral jejunal biopsy specimen. Monoclonal IgM with lambda chains was found in the serum and lambda chains in the urine. Analysis of post-mortem tissue by peroxidase-anti-peroxidase techniques provided some information on the cellular basis of this peculiar process.

Clinical History

A 54-year-old farmer's wife had experienced intermittent fever and flatulence since August 1978, and had also lost weight. On admission to hospital in November 1978 she had splenomegaly to the level of the umbilicus. She was pancytopaenic: haemoglobin 106 g/l, WBC 2.5×10^9/l with a slight relative lymphocytosis, platelet count 108×10^9/l. ESR was 32 mm/h and total serum protein 64 G/l. Liver function tests were normal. A barium meal and follow-through examination revealed a marked widening of the duodenal and jejunal mucosal folds. There were no fundal changes attributable to the hyperviscosity syndrome. The condition of the patient deteriorated quickly. She had pale-coloured stinking watery stools and increasing abdominal cramps. Based on the immunoglobulin studies, the barium meal and follow-through examinations and the peroral jejunal biopsy, a diagnosis of abdominal macroglobulinemia was reached and treatment with chlorambucil and prednisolone was started. The therapy had no effect on the patient's condition, which deteriorated steadily. A pleural effusion, ascites and generalized oedema appeared, diarrhoea and proteinuria increased, and the patient died on January 14, 1979.

Laboratory Investigations

The serum contained cryoglobulins. Serum immunoglobulins: S-IgG 5.3 g/l, S-IgA 1.1 g/l, S-IgM 14.5 g/l. Immunoelectrophoresis of the serum revealed a monoclonal IgM lambda immunoglobulin and also free lambda chains. There were also free lambda chains in the urine. Immunoelectrophoresis of the pleural effusion showed both monoclonal IgM lambda and free lambda chains. Vitamin A absorption was abnormally low, but the xylose test was normal. The Schilling test showed borderline absorption, 7%. The glomerular antibody test was negative, as also was the smooth muscle antibody test. There was a strong positivity in the mitochondrial antibody test (1:10,000), possibly caused by the mitochondrial antibody specificity of the component M. The complement components C_3 and C_4 had decreased, and serum cholesterol was very low (1.5 mmol/l), lipoprotein electrophoresis revealing only a weak beta fraction and no alpha-fraction. The plasma volume was increased, 63 ml/kg. The bone marrow aspirate revealed an increased number of normal-looking plasma cells and eosinophils with normal haemopoiesis. No lymphoreticular cells or mast cells indicating Walderström's macroglobulinemia were seen. Fine needle biopsy of the spleen also revealed a considerable increase in normal-looking plasma cells, but no changes pointing to Waldenström's disease. No extramedullary haemopoiesis was seen. X-ray examination did not disclose any lytic lesions in the skeleton.

Materials and Methods

The first peroral jejunal biopsy specimen and the postmortem tissue, were fixed for paraffin sectioning in 10% buffered formalin and processed by standard histology procedures for light microscopy. The following stainings were used: haematoxylin-eosin, Giemsa, alkaline Congo Red and crystal violet for amyloid, and hematoxylin-PAS with diastase. The second jejunal biopsy specimen was divided into portions for histology, immunohistochemistry and electron microscopy. Sudan IV stain was used on frozen sections for lipid demonstration.

Immunohistochemistry

a) Immunofluorescence (IF). Air-dried 5 μ cryostat sections were studied by direct immunofluorescence for the presence of IgG, IgM, IgA, C_3 and fibrin deposits using monospecific anti-immunoglobulin, anti-complement and antifibrin antisera fluorescein-isothiocyanate (FITC) conjugates. The conjugates, with F/P molar ratios of between 2.0–2.3, were from Behringwerke, Marburg. The presence of kappa and lambda chains was studied using specific anti-lambda and anti-kappa FITC conjugates. These conjugates, with an F/P molar ratio of 2.3, were from Dakopatts, Copenhagen.

Controls. Rabbit antisera against the various human immunoglobulins (IgA, IgG, IgM, kappa and lambda), C_3 and fibrinogen provided intrinsic controls one against the other. The reactions were also blocked by pretreatment with unconjucated antiserum. The blocking control was not entirely satisfactory, but this does not imply non-specificity (Sternberger 1979).

b) The peroxidase-antiperoxidase immuno-complex procedure (PAP) was performed as described by Sternberger (Sternberger 1979). Rabbit anti-immunoglobulin antisera specific for IgG, IgA, IgM and antisera for complement C_3 and fibrinogen were obtained from Dakopatts, Copenhagen and Behringwerke, Marburg. Anti-kappa, anti-lambda light chains antisera, swine anti-rabbit-serum-protein antiserum and peroxidase reagent were obtained from Dakopatts.

Controls. 1) Rabbit antisera against the various human immunoglobulins, and their components provided intrinsic controls one against the other. 2) Replacement of specific primary antiserum with PBS. 3) Replacement of specific primary antiserum with "normal" serum from the same species. This "normal" serum contained some reactivity for tissue components localized by specific antisera at low dilutions but not in working dilutions (1:1,000–1:16,000). 4) In order to check the specificity of the anti-light chain antisera, we applied the PAP techniques with lambda and kappa antisera to the post-mortem bone marrow tissue of an IgG kappa myeloma case.

Electron Microscopy

One millimeter cubes of the jejunal biopsy tissue were fixed in 1.5% glutaraldehyde in 0.1 M phosphate buffer and postfixed with 2% OSO_4 in phosphate buffer. Dehydration was carried out in acetone. The specimen was embedded in EPON 812 and sectioned on an LL B Ultrotome. The sections were stained with lead citrate and uranyl acetate and examined with JEM 100 C microscope.

Results

Autopsy Findings

A fresh infarct about one cm in diameter was found in the pons, but the intracranial tissues were otherwise unremarkable, as were the thyroid gland and upper respiratory tract. There was severe oedema in the lungs and about 200 ml bloody fluid in the pleural spaces. The hilar and mediastinal lymph nodes were moderately enlarged. The heart, aorta and vena cava superior were unremarkable. The stomach and colon, including the rectum, were normal. The entire small intestine was abnormal. The colour of the mucosa was greyish-white, the intimal folds were accentuated, blunt and swollen, the mesenteric lymph vessels were obliterated and yellowish white, and the mesenteric lymph nodes were moderately enlarged and their cut surfaces pale grey. The liver was slightly swollen, pale brown, showing no focal changes. The spleen was markedly enlarged, weighing 790 g, with a slightly rubberish consistency and

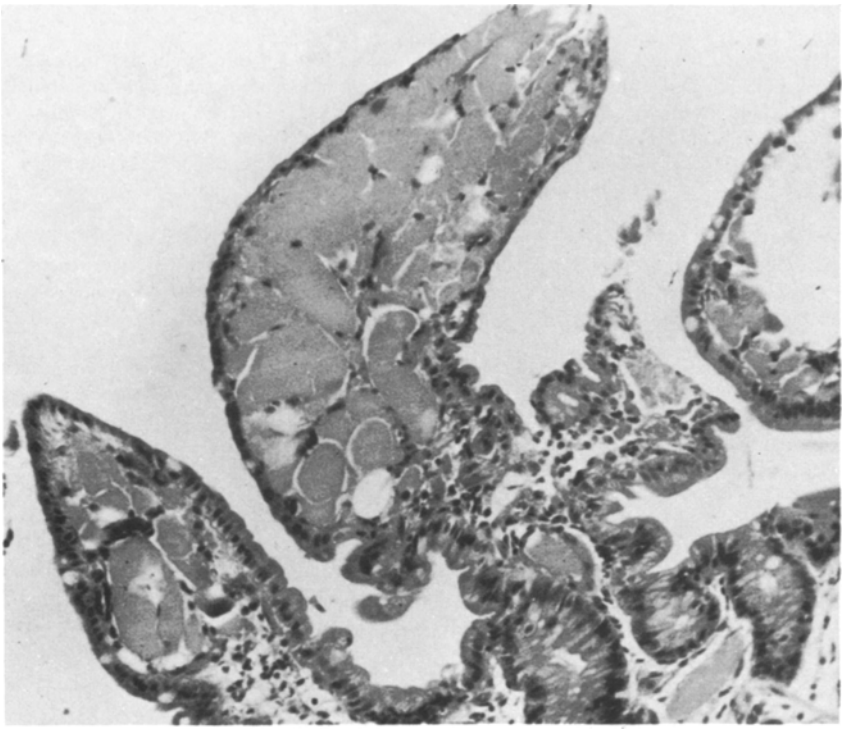

Fig. 1. Extracellular amorphous material in the intestinal lamina propria and dilated lymphatics. Haematoxylin-eosin × 160

rusty red cut surface. The renal cortex was slightly swollen, but the medulla was normal macrosopically. The lower urinary tract, genitalia, suprarenal glands, pancreas, and large retroperitoneal vessels and portal vein were unremarkable.

The bone marrow of the vertebrae contained no abnormal infiltrations macroscopically, but the femoral bone marrow showed areas which appeared to be slightly more extensive and softer than normal bone marrow. No bone erosions were found at autopsy.

Light Microscopy

Histological sections of the jejunal biopsy specimen and autopsy tissue showed an accumulation of extracellular, amorphous, eosionophilic, diastase-resistant PAS-positive material in the entire lamina propria of the whole of the small intestine and in the dilated intestinal lymphatics and mesenteric lymp nodes (Fig. 1). In these lymph nodes the material was packed into a compact mass which almost totally obliterated the lymphatic tissue. There were some histiocytes in the intestinal lamina propria, and also some plasma cells, but no tumor cells infiltrations were found. Dark staining, diastase-resistant, PAS-positive

amorphous extracellular material was also found in several visceral organs such as the spleen, thyroid gland, suprarenal gland, hypophysis, renal tubules and glomeruli and many small blood vessels, including the intracerebral vessels. The material gave a negative result in stains for amyloid.

The bone marrow, spleen, and mediastinal lymph nodes possessed scattered foci or loosely diffuse cellular infiltrations consisting of lymphocytes, plasma cells and plasmacytoid cells. A few of these cells contained globular, diastase-resistant PAS-positive material in their cytoplasms, and the same material was sometimes found in the interstitial tissue. Occasional large cells exhibited characteristics of immunoblasts or centroblasts. The general architecture of the lymph nodes was well preserved. There were few or no germinal centres, and those present were inactive and had their reticular meshwork and sinusoidal system well preserved. We were unable to find any "supranuclear windows" in the cells.

Immunohistochemistry

The direct immunofluorescence techniques applied to the frozen sections of the second jejunal biopsy specimen revealed a strong reaction with anti-IgM and anti-lambda antisera both in the lamina propria, lymphatics and in the epithelium (Fig. 2a and b). Anti-kappa antiserum exhibited a moderately strong reaction in the epithelial layer and also a weaker reaction in the lamina propria. Anti-IgA antiserum gave a slightly positive reaction on the epithelial surface. The reaction with anti-IgG antiserum was interpreted as negative. These techniques also pointed to small deposits of fibrin under the basement membrane of the intestinal epithelium and capillary walls.

The material which had accumulated in the intestinal lamina propria and intestinal lymphatics (pre-mortem and postmortem tissue) gave a strongly positive reaction with anti IgM and anti lambda antiserum when studied by the PAS techniques (Fig. 3a). The material in the intestinal lymphatics also gave a rather strong reaction with antikappa antiserum (Fig. 3b). The anti IgA and anti IgG antisera gave a very weak positive reaction, but one still clearly distinguishable from the control specimens, which were entirely negative.

When applied to the post-mortem spleen and bone marrow tissues, the PAP techniques gave the following results. A large majority of the plasmacytoid cells and plasma cells in the bone marrow gave a strongly positive reaction with sera against IgM and lambda and kappa light chains, although the reaction with anti-kappa antiserum was slightly weaker (Fig. 4). Some IgG positive cells were encountered. The findings for the spleen paralleled those for the bone marrow, with the exception that there were only a few cells stainable with the anti-kappa antiserum present which were still strongly reactive. Only a few cells reacting with anti-IgG antiserum were found.

The anti-IgA antiserum gave negative results in both the spleen and bone marrow tissue. The control slides for all the above tissues were entirely negative. The post-mortem bone marrow of a IgG kappa myeloma case presented an abundance of cells which gave a positive reaction with anti-kappa antiserum but not with anti-lambda antiserum.

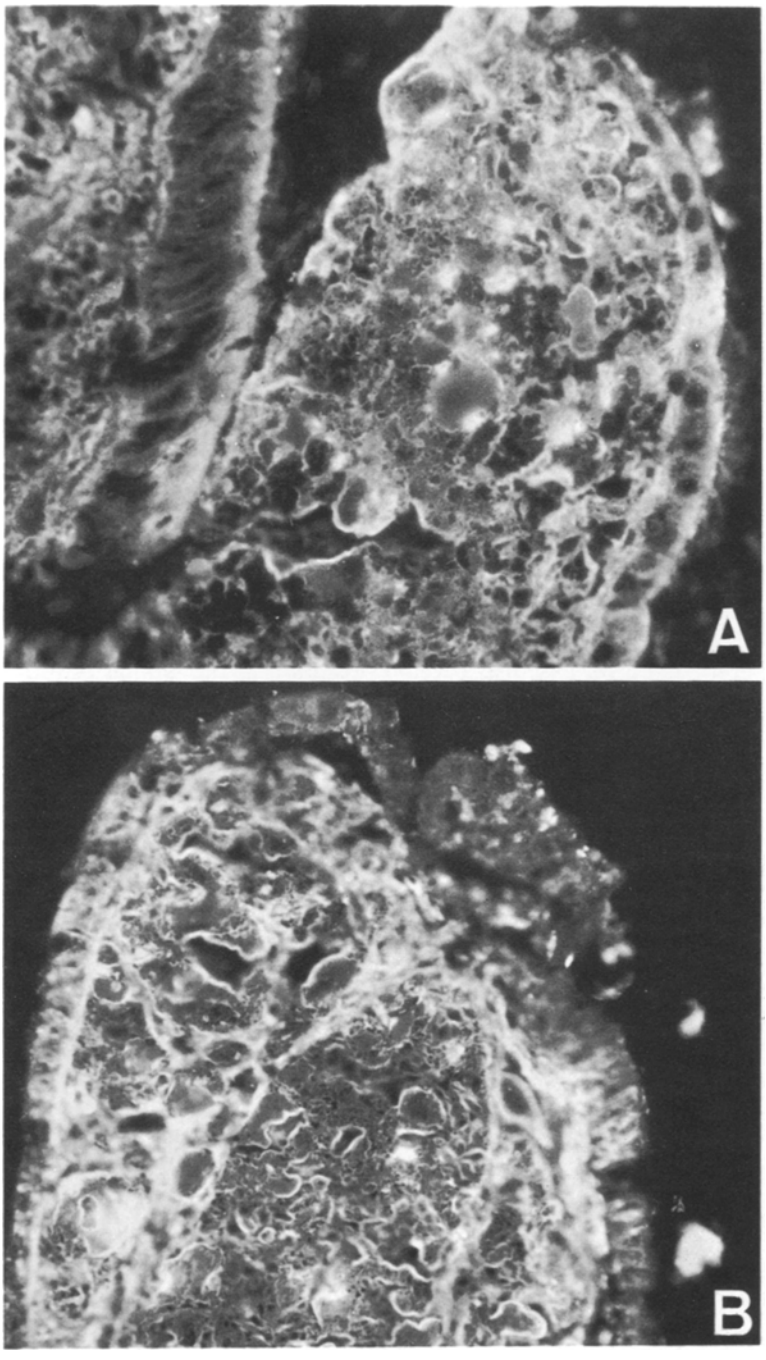

Fig. 2A and B. Direct immunofluorescence of the peroral jejunal biopsy tissue. There is a strongly positive reaction in the extracellular space of the lamina propria and dilated lymphatics, and also in the epithelial layer, with anti-IgM (**A**) and anti-lambda (**B**) antisera. × 200

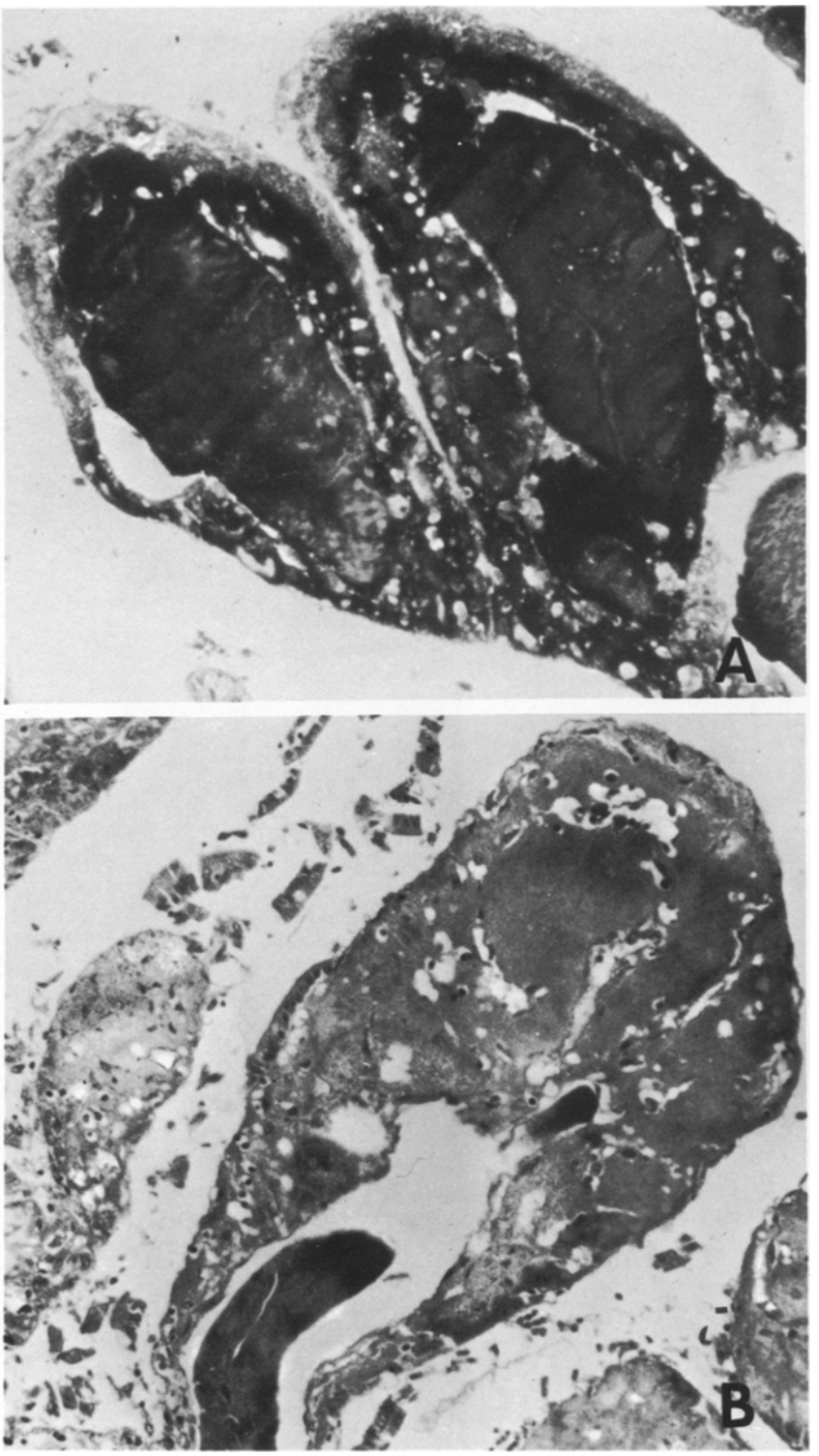

Fig. 3A and B. PAP techniques applied to the post mortem jejunal tissue. Anti-IgM antiserum (**A**) gives a strong positive reaction in the material deposited in the extracellular tissue and dilated lymphatics. Anti-kappa antiserum (**B**) gives a distinct positive reaction only in the lymphatics. $\times 200$

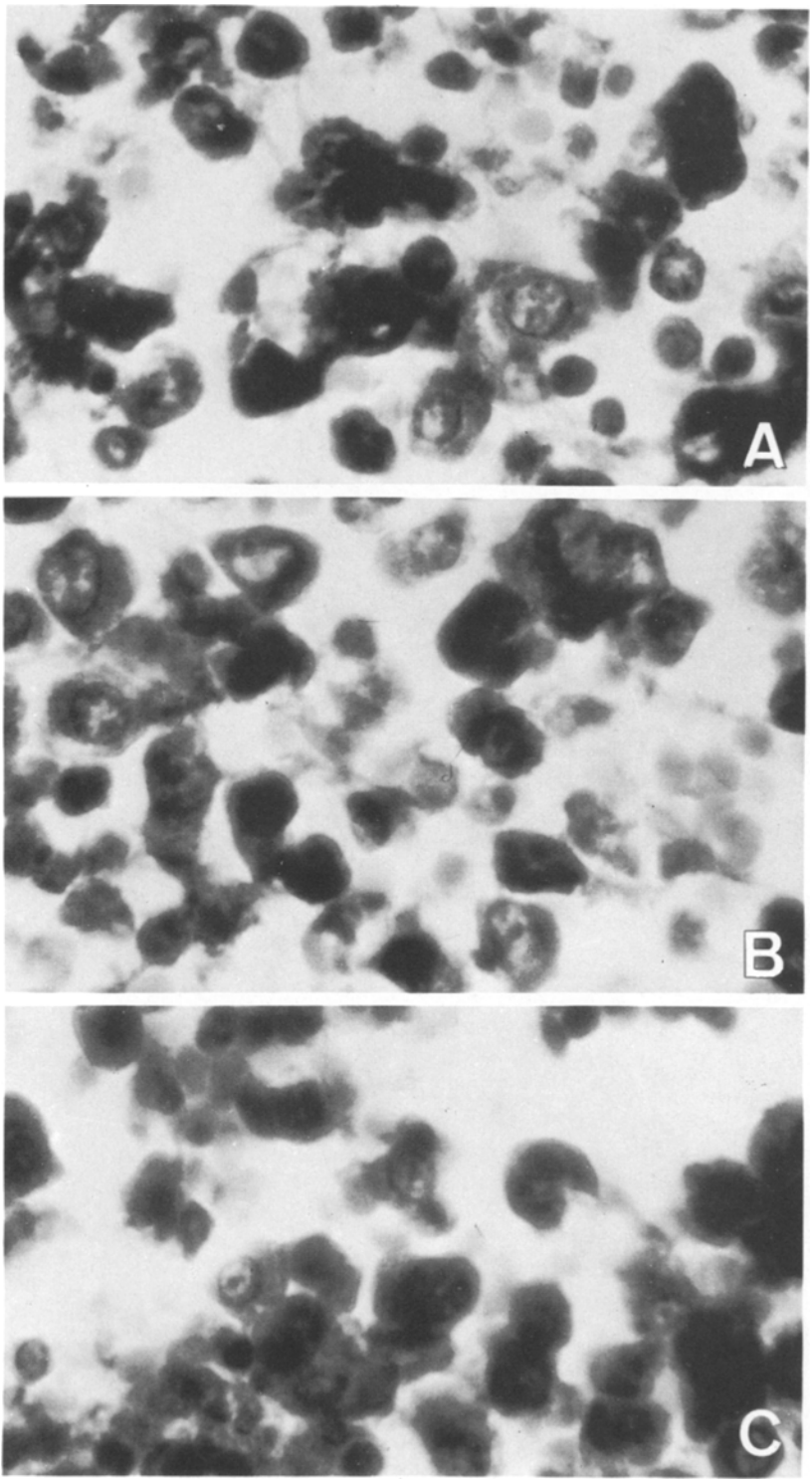

Fig. 4A–C. PAP techniques applied to the post-mortem bone marrow tissue. There is a distinct positive reaction in the plasma cells and plasmacytoid cells with anti-IgM (**A**), anti-lambda (**B**) and anti-kappa (**C**) antiserum. × 500

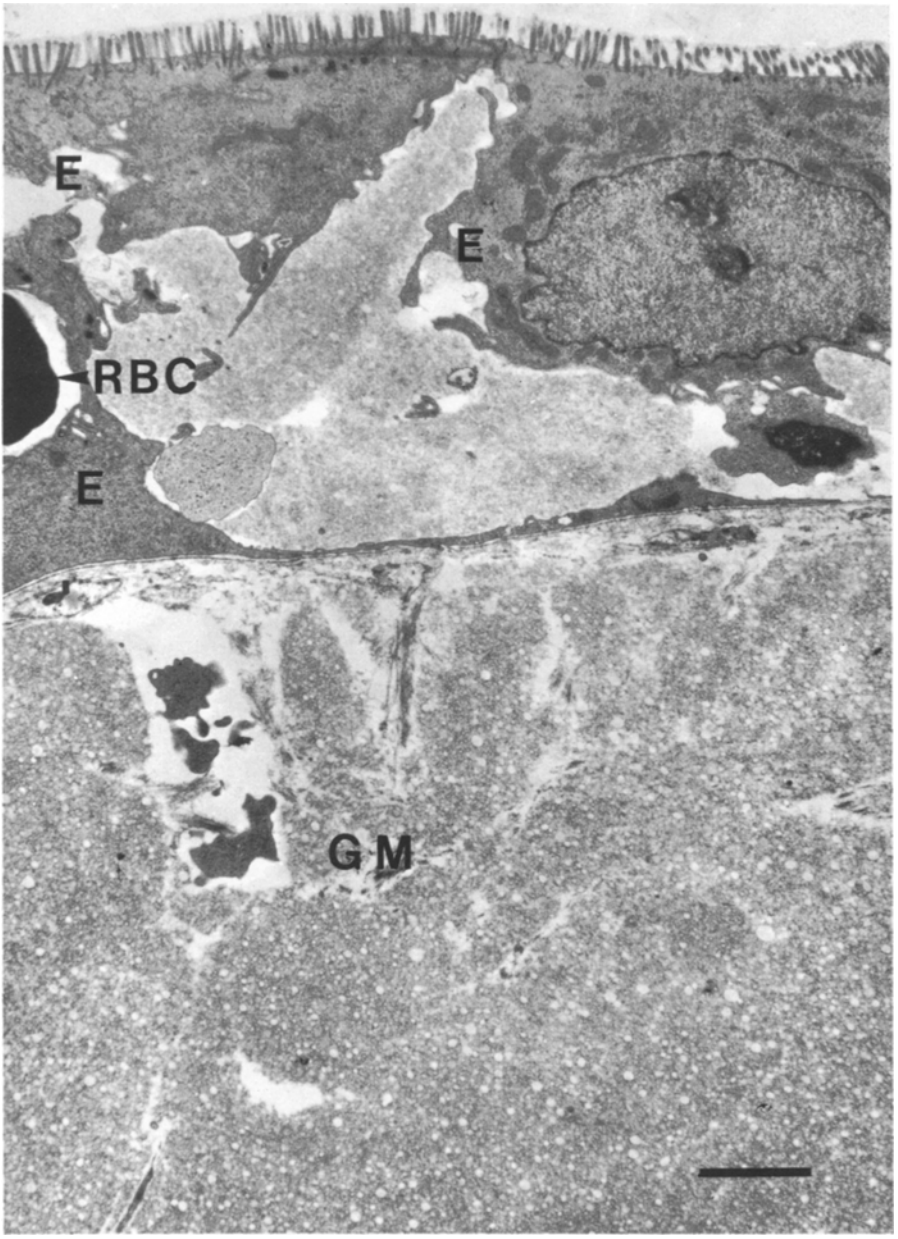

Fig. 5. Subepithelially located masses of globular stored material (*GM*), some cellular debris and infiltration of red blood cells (*RBC*) and material reminiscent of protein between the intestinal epithelial cells (*E*). Bar = 2 μm

Electron Microscopy

Electron microscopy of the peroral jejunal biopsy sample disclosed two components deposited extracellularly in the intestinal mucosa (Fig. 5), a granular electron-dense material and lipid droplets of varying size up to 4,000 Å. Some macrophages contained this in their cytoplasm, and it was also seen extracellularly above the basement membrane, where it seemed to push the epithelial cells apart. The particle size in the material between the epithelial cells appeared to be somewhat smaller, and there were some signs of disintegration of the material in this location.

Lipids

The very compact amorphous material, which appeared to be composed of immunoglobulins, did not stain with Sudan IV, although there was an abundance of Sudan IV-positive droplets in some places in the villi, where the accumulation of the immunoglobulins was looser.

Discussion

From the clinical standpoint, the present case fulfils the criteria for macroglobulinemia. The patient's serum contained monoclonal IgM with lambda chains, and free lambda chains were also detectable in the urine and pleural fluid. The malabsorption syndrome and its consequences dominated the clinical picture, but can be explained as part of the hyperviscosity syndrome (stagnation of the macroglobulin in the intestinal mucosa, lymphatics and mesenteric lymph nodes). It is also possible that the cerebral infarct was a complication of the hyperviscosity syndrome, caused by the cryo-macroglobulinemia.

The issue becomes more complicated when we come to the tissue and cellular level and raises questions concerning the concept of monoclonality and, of course, the specificity of the immunohistochemical methods used.

In the case of Pruzanski et al. (1973) the immunohistochemistry (IF) revealed only IgM (lambda) in the intestinal deposits, corresponding to the findings in serum. In our case, however, both IF and PAP techniques demonstrated a significant amount of kappa light chains in the deposits in addition to IgM and lambda light chains. It was also surprising to find a large number of cells which reacted with anti-kappa antiserum in the bone marrow and spleen, in addition to IgM and lambda light chain containing cells.

These findings may be explained by the cross-reactivity of the lambda and kappa antiserum. The following facts nevertheless argue against this assumption:

1. The Dakopatts antisera have repeatedly been proved to be of a high specificity by other investigators (e.g., Taylor et al. 1978).

2. The antisera gave a clear-cut positive reaction within the dilution range of 1:1,000–1:16,000 in our experiments. When the specific primary antiserum was replaced with "normal" rabbit serum some reactivity was detected but

only at low dilutions (< 1:1,000). This is in accordance with the findings of De Lellis et al. (1979).

3. In a case of IgG kappa myeloma, the tumor cells in the post-mortem bone marrow tissue exhibited a strong positive reaction with anti-kappa antiserum but no reaction with anti-lambda antiserum.

There remains the possibility that a cleavage of antigens in post-mortem autolysis and/or during various steps in the peroxidase procedure might yield fragments common to several determinants. The "discrepancy" between the serum findings obtained by the precipitation method and the tissue and cellular findings obtained by the immunohistochemical techniques may nevertheless be a matter of a difference in sensitivity between the methods and our case possibly represents an example of the broad range of immunochemical findings in B-cell proliferations (Taylor et al. 1978; Lennert et al. 1978). Taylor et al (1978) have recently described four myeloma cases in which only one type of light chain was found in the serum, but cells containing both kappa and lambda chains were present in the bone marrow. Two of these cases were designated as "poorly differentiated" myelomas. In our case, some areas in the cellular infiltrates in the spleen and bone marrow seem to correspond to the polymorphic subtype of LP immunocytoma in the Kiel classification (Lennert et al. 1978). We also suggest that our case represents the splenomegalic variant within the clinical spectrum of LP immunocytomas.

In electron microscopy, the material in our case exhibited the presence of lipid admixtured with the protein, as in the case of Pruzanski et al. (1973). The precise nature of this lipid remains open, since we used only Sudan IV as a lipid stain. Electron microscopy also showed an accumulation of abnormal extracellular material between the intestinal epithelial cells. This could be explained as a consequence of damage to the basal membrane, probably due to the pressure of the material which had accumulated in the lamina propria. The smaller particle size and signs of disintegration in the extracellular material above the basement could be a result of the enzymatic function of the intestinal epithelium.

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