A case of γ 3 heavy chain disease with vacuolated plasma cells: a clinical, immunological, and ultrastructural study

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SUMMARY A patient with lambda Bence-Jones proteinuria, Waldenström's macroglobulinaemia, and Franklin's disease (γ HCD), but without clinical evidence of a lymphoproliferative disorder, is presented. The serum contained two distinct immunoglobulin abnormalities: a monoclonal immunoglobulin M (IgM) of lambda type, and a protein fragment which was immunologically related to immunoglobulin G (IgG) and devoid of light chain activity. This γ HCD protein belongs to the γ 3 subclass with a molecular weight of approximately 60 000 daltons. The urine contained a Bence-Jones lambda protein as well as the γ HCD fragment. The two paraproteins were probably secreted by two different malignant clones. Ultrastructural study revealed pathological vacuolated plasma cells of a sort that has hitherto been principally described in association with μ HCD. The mechanism of the intracellular storage of pathological immunoglobulins is discussed in the light of the ultrastructural study.

The plasma cell dyscrasias, multiple myeloma and macroglobulinaemia, and the association between them have been the subject of intensive investigations in recent years.

The diagnosis of gamma heavy chain disease (γ HCD), described by Franklin *et al.* in 1964, requires the identification in serum and urine of homogeneous populations of incomplete heavy chains of the IgG immunoglobulin class. Up to 1973, more than 30 cases of γ HCD had been identified (Frangione and Franklin, 1973).

The γ HCD has a well-defined clinical and pathological picture. Generalised lymphadenopathy, enlargement of the liver and spleen, oedema of the soft palate and uvula, pleural effusion, and ascites have been described (Osserman and Takatsuki, 1964; Delmas-Marsalet *et al.*, 1971; Seligmann, 1972; Bloch *et al.*, 1973). The importance of the presence of atypical forms of plasma cells and lymphocytes is reported by most authors (Zucker-Franklin, 1964; Fisher and Zawadzki, 1970; Hobbs, 1971; Lennert *et al.*, 1975), and ultrastructural study has revealed abnormalities associated with the intracellular storage of pathological immunoglobulins (Feremans *et al.*, 1978).

Progress has also been made in defining the molecular mechanism of some special associations of paraproteins such as μ chains with IgG gammopathy (Josephson *et al.*, 1975), γ 3 chains with IgG 3 λ myeloma, as recently described by Adlersberg *et al.* (1978), or γ 1 heavy chain with IgG λ and free λ chains (Isobe and Osserman, 1974).

In this paper we describe an additional variant: a patient presenting the characteristics of Bence-Jones proteinuria, Waldenström's macroglobulinaemia, and γ HCD but without clinical signs of lymphoma or myeloma.

Material and methods

CASE REPORT

An 87-year-old white woman was admitted to St Pierre Hospital, Brussels, in September 1976 suffering from anaemia and lung congestion with heart failure. Before admission the patient had been in good health.

Physical examination

Physical findings on admission included a pulse rate of 140/min and a blood pressure of 190/110 mmHg and the patient appeared to have lost weight. There was bilateral lung congestion. She had no fever, no

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lymphadenopathy, no hepatosplenomegaly, and no palatal oedema.

Laboratory data

ESR 132 mm/h, haemoglobin 8.6 g/dl, RBC $2.7 \times 10^{12}/l$, haematocrit 29.7%, reticulocyte count 1.5%, MCV 96 fl, MCHC 31.2 g/dl, MCH 30 pg, WBC $8.6 \times 10^9/l$ with 51% lymphocytes, 47% neutrophils, 2% eosinophils, 2% monocytes, platelet count $364 \times 10^9/l$, iron 16 $\mu\text{mol}/l$ (89 $\mu\text{g}/100$ ml), IBC 52 $\mu\text{mol}/l$ (288 $\mu\text{g}/100$ ml), haptoglobin 1.2 g/l (120 mg/100 ml). Bone marrow aspiration showed 19% dysplastic plasma cells (nucleocytoplasmic asynchronism and some cells resembling reticulum cells) and 22% lymphocytes, with a normal cellularity. Serum glutamic oxaloacetic transaminase and glutamic pyruvic transaminase gave, respectively, 21 and 14 Karmen units (normal value 22), lactic dehydrogenase 171 Berger-Broida units (normal 200), gamma glutamyl transferase 78 IU (normal 28), creatinine phosphokinase 17 IU (normal 50), alkaline phosphatase 216 IU (normal 110), bilirubin 9 $\mu\text{mol}/l$ (0.5 mg/100 ml), cholesterol 4.3 mmol/l (167 mg/100 ml), triglycerides 1.1 mmol/l (98 mg/100 ml), urea nitrogen 88 mmol/l (123 mg/100 ml), creatinine 230 $\mu\text{mol}/l$ (2.6 mg/100 ml), calcium 2.1 mmol/l (8.4 mg/100 ml), phosphate 1.6 mmol/l (5 mg/100 ml), uric acid 8.8 mg/100 ml, sodium 130 mmol/l, potassium 4.4 mmol/l, chloride 97 mmol/l, and carbon dioxide 2.7 kPa (20 mmHg).

Chest x-rays revealed cardiomegaly and vascular lung engorgement but no mediastinal lymphadenopathy. Liver-spleen scanning showed no hepatosplenomegaly; on an electrocardiogram there was sinus tachycardia with left bundle-branch block without myocardial infarction. We found small kidneys with a delay of bilateral elimination after intravenous pyelography.

Immunological data (Table)

The total serum protein was 80 g/l (8 g/100 ml). Microzone electrophoresis of serum and urine revealed migrating paraproteins which were further

characterised by immunoelectrophoresis. Cryoglobulin, LE cells, antinuclear factor, rheumatoid factor, and VDRL tests were negative. Direct Coombs' test was weakly positive for IgG. Proteinuria was 0.2 g/l. Quantitative examination of serum immunoglobulins gave the following results: IgG 60 g/l (6000 mg/100 ml), IgM 13.5 g/l (1350 mg/100 ml) and IgA 2.5 g/l (250 mg/100 ml).

The distribution of chain specific surface immunoglobulins was γ 10% (normal 2.5%), α 0.4% (normal 0.4%), and μ 6% (normal 5-10%). The K: λ ratio was 1:1.

Treatment and course

Lung congestion was successfully treated with diuretics and digoxin. Anaemia was corrected by red cell transfusion. In July 1977 (10 months after diagnosis) the patient died of pneumonia. Post-mortem examination was refused.

METHODS FOR PROTEIN STUDIES

Microzone electrophoresis of the patient's serum and concentrated urine was performed on cellulose acetate strips in a pH 8.6 veronal buffer. Immunoelectrophoresis was done according to Grabar and Williams (1953) and Osserman and Lawlor (1961), using polyvalent antisera against whole serum and specific antisera to heavy and light chains from different companies (Kallestad Lab, Chaska, Minn, USA; Behring Diagnostic Marburg, RFA; Dakopatts, Copenhagen, Denmark; Netherlands Red Cross Blood Transfusion Service, Amsterdam, Holland). Agarose gel electrophoresis was performed according to Johansson (1972) and immunofixation according to Ritchie and Smith (1976). Serum concentrations of immunoglobulins were measured by radial immunodiffusion (Mancini *et al.*, 1965) using antibody-containing agar plates purchased from Kallestad Lab, Chaska, Minn, USA. Molecular weight was determined by dodecyl sulphate acrylamide gel electrophoresis using protein markers of known size obtained from Boehringer, Mannheim, RFA, according to Weber and Osborn (1969) and by Sephadex gel filtration on calibrated columns (Andrews, 1965). The identification of γ heavy chain as belonging to subclass 3 was performed according to the directions for use of anti-human IgG subclass antisera by the Mancini technique (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service).

METHODS FOR CELLULAR STUDIES

Light microscopy

Bone marrow smears were routinely stained by the May-Grünwald Giemsa method.

Table Immunological data

Serum protein electrophoresis	80 g/l
albumin	43 %
α_1 -globulin	2.2 %
α_2 -globulin	7.7 %
β -globulin	7 %
inter β - γ peak	27.8 %
γ -globulin	12.3 %
Immunoelectrophoresis:	
heavy chain disease of γ G3 specificity	
proteinuria with λ light chains	
IgM lambda monoclonal component	

Fluorescent microscopy (Pearse, 1960)

Smears were fixed for 5 minutes in absolute ethanol and rinsed in 0.15 M pH 7.4 phosphate buffer at room temperature. Dako rabbit antihuman fluorescein conjugated antibodies, for IgA, IgM, IgG specific for Fc fragment, K, λ , were used at the dilution of 1/40 at 37°C.

Electron microscopy

Bone marrow obtained by sternal puncture was immediately fixed for 30 minutes at room temperature in 4% distilled glutaraldehyde in 0.1 M phosphate buffer.

The cells were rinsed in the same buffer and post-fixed with 2% osmium tetroxide (Millonig, 1962). Dehydration took place in graded concentrations of ethanol with centrifugation for 10 minutes at 1000 rpm at each step of the manipulation.

Finally, the material was embedded in Epon (Luft, 1961) and sectioned with a Diatome diamond knife on an LKB Ultratome III microtome. Ultrathin sections were stained with uranyl acetate and lead and observed with a Siemens Elmiskop I electron microscope.

Results

Fig. 1 Agarose gel electrophoresis of the patient's serum (se) and $\times 100$ concentrated urine (ur). The M-components are clearly visible in the β - γ region.

PROTEIN STUDIES

Microzone electrophoresis revealed a broad peak of β - γ mobility for serum and concentrated urine. Agarose gel electrophoresis showed a second peak in serum and urine (Fig. 1). Quantitative serum immunoglobulin analyses gave values of about 60 g/l (6000 mg/100 ml) for IgG, 13.5 g/l (1350 mg/100 ml) for IgM, and 2.5 g/l (250 mg/100 ml) for IgA. Immunoelectrophoretic patterns at various dilutions of the patient's serum demonstrated the presence of an abnormally slow migrating arc in the β - γ region (Fig. 2).

The slow migrating protein band reacted with whole IgG and Fc fragment antisera but not with anti-k or anti- λ sera nor with antisera specific for α chain, μ chain, and Fab fragment (Fig. 3). The molecular weight of the Fc fragment was estimated to be about 60 000 daltons.

In addition, a second band reacted with μ and λ chain specific antisera, as seen by immunofixation in Figure 4. Immunoelectrophoresis performed on a 100-fold concentrated urine sample showed the slow migrating fragment reacting identically with that in the serum. A second distinct band reacted only with free λ light chain antisera (Fig. 5). No precipitation arc was detected between Fab specific anti-serum and the concentrated urine sample.

CELLULAR STUDIES

The reaction with anti-IgM and anti- λ fluorescein-

Fig. 2 Immunoelectrophoresis of the patient's serum (sp) undiluted (1/1), diluted 1/3 and 1/9 in central wells of each slide compared with normal human serum (shn), undiluted (1/1), diluted 1/3 and 1/9 in exterior wells. The slides are developed with a polyspecific antiserum antinormal human serum.

Fig. 3 Immunoelectrophoresis of the patient's serum. A broadly dispersed precipitation arc is demonstrated in the serum developed with anti-IgG antiserum (ra/IgG) and anti-Fc fragment antiserum. This arc is not developed with anti-Fab antiserum nor with anti-K and anti- λ antisera (ra/K ch; ra/ λ ch).

Fig. 4 Immunofixation analysis of the patient's serum. A fine reacting band with the anti-IgM antiserum and the λ light chain antiserum is clearly identified. The absence of the K band is strongly suggestive of a IgM λ monoclonal component.

conjugated antisera was positive in many lymphocytes. Fc fragment antiserum gave a marked intracytoplasmic fluorescence in a few plasma cells. Several lymphocytes appeared with intranuclear inclusions limited by a single membrane contrasting with the two-layer membrane of the nuclear blebs.

Fig. 5 Immunoelectrophoresis pattern of the $\times 100$ concentrated patient's urine (up) in the central wells; normal human serum in exterior wells (shn). The top slide is developed with anti-normal human serum and reveals two abnormal components. The first is the Fc fragment as correlated by agarose gel electrophoresis and the second a Bence-Jones λ light chain reacting with anti- λ antiserum (ra/ λ ch).

Sometimes a nuclear body with ribosome-like structures was seen near these inclusions (Figs 6-8).

Nucleoli were often observed. Some lymphocytes presented cytoplasmic granules surrounded by a clear zone. Patterns of cytoplasmic microfilaments were also encountered. The plasma cells showed a variable dilatation of rough endoplasmic reticulum (RER) cisternae containing electron-dense proteinaceous material. There was a well-developed Golgi apparatus. A few plasma cells presented the aspect of 'storage cells' with an extreme expansion of the RER cisternae. Clasmotosis with release of cytoplasmic fragments containing RER cisternae was also encountered. A very striking feature was the presence of cytoplasmic vacuoles of variable size inside many plasma cells. These vacuoles frequently contained membrane fragments which appeared to collapse away from their limiting membrane. More often located near the Golgi apparatus, they sometimes seemed to be in continuity with broken cisternae of RER (Figs 9-11). It was not unusual for vacuoles to extend to the periphery of the plasma cell where defects in the plasma membrane occurred.

Discussion

The main interest of this case is the association of γ 3 HCD and Waldenström's macroglobulinaemia. The laboratory and pathological data, with the exception of serum and urine proteins, were non-specific. Anaemia and heart failure were the only striking features.

Fig. 6 Two intranuclear inclusions (arrows) in a bone marrow lymphocyte. $\times 1200$

Fig. 7 Similar intranuclear inclusion (I) in a bone marrow lymphocyte. $\times 8000$

There was no abrupt change in the clinical condition of the patient as described by Spengler *et al.* (1972); indeed the initial paraproteins persisted unchanged throughout the period of observation.

Physicochemical data published on γ 3 HCD explain some of our observations (Fine *et al.*, 1968; Keller *et al.*, 1970; Isobe and Osserman, 1974; Josephson *et al.*, 1975; Virella *et al.*, 1977; Creysse

Fig. 8 High magnification of a lymphocyte showing an intranuclear inclusion (I), a nuclear bleb (P), and a nuclear body with ribosome-like material (B). $\times 44\,000$

Fig. 9 A vacuolated plasma cell. $\times 1125$

et al., 1977; Adlersberg *et al.*, 1978). The amounts of IgG and IgM were always greater than the value calculated for the fraction of the serum protein on the basis of the total protein concentration and

the fractional values obtained from the microzone electrophoresis scan. These findings suggest an IgG fragment diffusing through the gel at a considerably faster rate than normal 7 S IgG.

Fig. 10 A plasma cell with a large cytoplasmic vacuole: N = nucleus, γ = disrupted membrane, Z = membrane fragments, c = centriole, G = Golgi apparatus, R = rough endoplasmic reticulum, M = mitochondria. $\times 21\,230$

Definitive diagnosis was based on the demonstration in serum and urine of a broad spike with inter β - γ mobility, reactive with antisera to heavy chains but not light chains. The immunoelectrophoretic analyses clearly established the presence of the Fc fragment associated with failure of light chain production. There was no evidence of a spontaneous cleavage of the monoclonal IgG into its constituent Fc and Fab fragments.

The results of the immunofixation studies showed the monoclonality of the IgM confirmed by the strong monospecificity of the antiserum; when tested by bidimensional electrophoresis and some experiments performed with purified IgM fraction isolated by preparative polyacrylamide gel electrophoresis, other immunoglobulin classes cannot be detected.

From these observations different questions can

Fig. 11 High magnification shows a communication between RER cisternae and a vacuole (arrows). $\times 36\,000$

be considered. Are the two paraproteins secreted by one or two cellular clones or by a submutation of the malignant clone previously synthesising IgM or γ 3 heavy chains, as suggested by Virella *et al.* (1977)? Further experiments on their chemical structure would be necessary to classify this γ HCD protein into one of the groups described by Franklin (1978), in other words, to determine the position of the aminoacid deletion.

A study of the transcription and the translation of DNA is needed to define the mechanism responsible for these deletions. A messenger RNA smaller than that for an intact heavy chain (Buxbaum and Alexander, 1977), the probability that more than one gene may be involved in the regulation of heavy and light chain synthesis, and the existence of regulatory defects in the cell (Franklin, 1978) have been suggested.

In addition to this defect, the presence of other immunoglobulin variants in serum and urine may be ascribed to a plasma cell neoplasm which may or may not be related to the heavy chain disease.

The haematological pattern was characterised by lymphocytosis in the blood and by a slightly increased number of lymphocytes and plasma cells in the bone marrow with many morphological abnormalities. The lymphocytes had nuclear pockets, intranuclear inclusions, nuclear bodies, and several nucleoles. Their cytoplasm was relatively abundant with some RER cisternae and occasionally patterns of microfilaments, as is often seen in neoplastic lymphocytes (Zucker-Franklin, 1975). The nuclear blebs correspond to cytoplasmic invaginations inside the nucleus. The most striking lymphocyte abnormalities were the presence of intranuclear inclusions and a few nuclear bodies. The intranuclear inclusions limited by a monolayer membrane were probably formed by invagination of the inner nuclear envelope. Their origin has not yet been elucidated; some authors attribute to them a cytoplasmic, others a nuclear, origin (Bessis *et al.*, 1963; Brittin *et al.*, 1963; Kuhn, 1967). Such inclusions are characteristic of Waldenström's disease. They can be seen in myeloma cells and also in plasma cells in inflammatory conditions. Immunofluorescent techniques have revealed the presence of IgM inside similar inclusions (Solomon *et al.*, 1963). The nuclear bodies and their ribosome-like particles were described near intranuclear inclusions in cases of macroglobulinaemia by Kuhn (1967); his work supports the intranuclear origin of these inclusions.

Spectacular morphological changes were also detected in the plasma cell line. Plasmacytes showed all intermediate stages of immunoglobulin accumulation. 'Flaming cells' and 'storage cells' represented the extreme degree of this accumulation (Fisher and Zawadzki, 1970; Maldonado *et al.*, 1965). The clasmatosis phenomenon, as in the myeloma cells, indicated secretion of pathological immunoglobulins (Maldonado *et al.*, 1965). But the most unusual feature for γ HCD was the presence of cytoplasmic vacuoles of different sizes. This appearance is characteristic of μ HCD (Ballard *et al.*, 1970; Forte *et al.*, 1970; Franklin, 1975; Jonsson *et al.*, 1976), except for the observation of several cytoplasmic vacuoles on light microscopy in the first case of γ HCD (Franklin *et al.*, 1964). Similar vacuoles were also reported by Aula *et al.* (1975) inside lymphocytes in lipidic lysosomal disorders. The ultrastructural analysis of vacuolated plasmacytes agrees with the descriptions of Zucker-Franklin in a case of μ HCD (Zucker-Franklin and Franklin, 1971). The vacuoles were indeed limited by a wavy disrupted membrane and contained membrane fragments. As Zucker-

Franklin has noted, the vacuoles are not due to dilatation of RER, but at high magnification a continuity between the vacuoles and RER cisternae may be observed.

In conclusion, unusual findings encountered in this case are, on the one hand, the association of three abnormal proteins and, on the other, the presence of vacuolated plasma cells typical of μ HCD.

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