Demonstration of serum monoclonal immunoglobulin in a case of non-secretory myeloma by immunoelectrophoresis

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SUMMARY A paraprotein band in non-secretory myeloma was shown using a highly sensitive technique of immunoelectrophoresis. The published work concerning non-secretory myeloma is briefly reviewed.

Non-secretory myeloma is a rare variant of multiple myeloma in which conventional agarose zonal and immunoelectrophoretic techniques fail to show a paraprotein in either serum or urine. We describe a case in which the highly sensitive technique of immunoelectrophoresis showed a small IgG κ monoclonal band in the patient's serum and discuss the implications of this finding.

Case report

A 54 year old woman presented in September 1982 with a one year history of exertional dyspnoea, a three month history of pain in the left hip, and spontaneous bruising of the limbs of recent onset. There was pallor and extensive bruising on both lower limbs, but no lymphadenopathy, hepatosplenomegaly, or sternal tenderness. Her haemoglobin concentration was 5.6 g/dl, white cell count $3.7 \times 10^9/l$, and platelet count $171.0 \times 10^9/l$. The blood film was leucoerythroblastic with 36% blast cells and the erythrocyte sedimentation rate was 83 mm in the first hour. A marrow aspirate showed almost total replacement by primitive cells resembling lymphoblasts (Fig. 1); however, Sudan black, periodic acid Schiff, acid phosphatase, chloracetate esterase, and non-specific esterase stains were all negative. Radiological skeletal survey showed lytic lesions in the right fifth rib, left ischium, and both proximal femora. Ultrasound examination of the abdomen showed no lymphadenopathy or organomegaly. Protein electrophoresis performed on a cellulose monoacetate strip was negative, as was agarose zonal electrophoresis and immunoelectrophoresis. There was

no Bence Jones proteinuria. There was hypogammaglobulinaemia with an IgA concentration of 0.4 g/l, IgG 5.4 g/l, and IgM 0.2 g/l (normal 0.9-4.5 g/l, 8-18 g/l, and 0.7-2.8 g/l).

A provisional diagnosis of acute undifferentiated leukaemia was made and chemotherapy comprising daunorubicin, vincristine, and Prednisolone was started. After one pulse of chemotherapy 20% plasma cells were found in the peripheral blood and a repeat marrow aspirate showed the typical morphological features of myeloma (Fig. 2).

Repeat zonal agarose electrophoresis and immunoelectrophoresis were negative and, again, no Bence Jones proteinuria was found. Immunoperoxidase studies of both bone marrow aspirates showed a monoclonal κ light chain staining pattern with a complete absence of demonstrable heavy chain production (Fig. 3). Immunoelectrophoresis^{1,2} performed on a blood sample taken after the first pulse of chemotherapy showed a small concentration of monoclonal IgG κ (Fig. 4). In view of this finding the immunoperoxidase studies were repeated after trypsinisation of the marrow sections and monoclonal IgG and κ chains were clearly shown in most of the malignant cells (Fig. 5).

Six pulses of combination chemotherapy according to the M₂ protocol³ were given, but marrow toxicity was unacceptably severe and treatment was changed to intravenous cyclophosphamide (1 g/m²) at three weekly intervals. The serum paraprotein remained demonstrable but cannot currently be quantitated by immunoelectrophoresis. The symptomatic response, however, was dramatic and 13 months after diagnosis the patient remains well and free from bone pain.

Fig. 1 Marrow appearances at presentation showing numerous immature cells resembling lymphoblasts. $\times 800$.

Fig. 2 Marrow appearances after chemotherapy showing typical morphological features of myeloma. $\times 800$.

Discussion

In about 1–2% of cases of multiple myeloma conventional zonal and immunoelectrophoretic techniques fail to show a paraprotein band in either serum or urine.^{4,5} Such non-secretory variants of myeloma seem to be unusually aggressive, with a reported median survival of seven and a half months in one series.⁶ The generally poor prognosis of non-secretory myeloma has been confirmed by other

authors.^{5,7–9} The absence of a detectable paraprotein in such cases also makes the monitoring of response to treatment more difficult.

Hobbs⁷ suggested that zonal and immunoelectrophoresis will detect a paraprotein in myeloma only when at least 20 g of tumour is present. Isoelectric focusing seems to be the most sensitive method available for showing the microheterogeneity or homogeneity of proteins, and when combined with immunofixation it may identify small paraprotein

Fig. 3 Marrow stained with immunoperoxidase showing a number of cells (arrows) staining positively with anti- κ . $\times 800$.

Fig. 4 Comparison of zonal and immunoelectrophoresis (IEP) with immunoelectrophoretic focusing (IIEF). Antisera in troughs are indicated to the right of the immunoelectrophoresis pattern. The numbers of immunoelectrophoretic focusing of the tracks indicate the process each received. Track 1 = trichloroacetic acid fixed to show total protein; track 2 = anti-IgG treated; track 3 = anti- κ treated; track 4 = anti- λ treated. Arrows denote the paraprotein position. N = normal human plasma; P = patient's serum.

Fig. 5 Trypsinised marrow sections stained with immunoperoxidase showing a number of cells (arrows) staining positively with anti-IgG. $\times 800$.

bands when conventional techniques have failed, as in the present case.¹

The explanation for the absence of detectable paraprotein in rare cases of myeloma has been the subject of considerable debate. In some cases intracellular immunoglobulin has been shown by immunofluorescence,^{10,11} implying a defect in immunoglobulin secretion rather than production, while in others no intracellular immunoglobulin was found.^{8,11} Putham and Miyake suggested¹² that the malignant cells in some cases either manufacture no immunoglobulin or produce an aberrant immunoglobulin not detectable using conventional antisera.

The finding of amyloidosis in one case of non-secretory myeloma raises the possibility that in some cases the malignant cells may secrete immunoglobulin in quantities too small to be detected by conventional techniques.⁶ In vitro secretion of small quantities of immunoglobulin has recently been shown in a case of non-secretory myeloma.¹³ While we are unable to prove conclusively that the paraprotein in the case reported here represents the secretory product of the malignant cells, the presence of intracellular immunoglobulin of identical heavy and light chain type strongly supports this contention. Further studies which may clarify this point, entailing the use of idiotypic antisera, are underway.

A report by Cereda *et al*¹⁴ highlights one of the problems associated with non-secretory myeloma, in which, by definition, a serum or urinary paraprotein cannot be identified. In their case an erroneous diagnosis of breast carcinoma was made partly on

the basis that biochemical markers of myeloma were absent; an IgA λ paraprotein was eventually identified using conventional techniques. Treatment for myeloma was then started, but the patient became profoundly neutropenic and died. It is possible that immunoelectrophoretic focusing could provide a prompt and definitive diagnosis of multiple myeloma in such difficult cases.

The detection of a small quantity of serum paraprotein in a case of non-secretory myeloma raises important questions about the biology of this disease and highlights the potential value of immunoelectrophoretic focusing as a diagnostic aid. We hope that with the development of reliable quantitative methods this highly sensitive technique will find a place in the biochemical monitoring of response to treatment.

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