Section of Clinical Immunology and Allergy

President Sir Michael Woodruff FRCS FRS

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Paraproteinaemia [*Abridged*]

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Paraproteins

Paraproteins typically appear as narrow bands after electrophoresis. Thanks to the concepts of Waldenström and Burnet and the fundamental work on immunoglobulin structure of Porter and others (reviewed by Hobbs 1966) there is now good support for the hypothesis that paraproteins are monoclonal immunoglobulins, and recently Marchalonis & Nossal (1968) showed that the antibody produced by a single cell indeed ran as a narrow band on electrophoresis.

Five classes of immunoglobulin are now known. Each 'has a basic unit of two heavy chains joined to two light chains. The classes can be identified using antisera specific to their heavy chains, γ, α, μ, δ or ϵ. Two major types of light chain are known, types κ and λ, but these are common to all classes. However, within a given molecule, the light chains are either κ or λ; hybrids cannot be found or made. The normal mixture of immunoglobulins is such that 70% of the molecules are type κ and 30% are type λ.

The clone of cells derived from the proliferation of a single cell would all be expected to produce identical molecules of immunoglobulin, which would therefore have identical electrophoretic mobility and run as a narrow band. In practice even artificial single haptens tend to evoke the proliferation of more than one clone of cells, though occasionally a narrow band response is found initially. Single proteins can have many antigenic sites. Micro-organisms have a wide spectrum of antigens and thus evoke an even wider spectrum of antibodies. Each antibody has its own particular structure and electrophoretic mobility and collectively a natural response to infection is seen as a broad increase in immunoglobulins usually representing all classes with heavy chains and light chains of all types and with electrophoretic mobilities from the inter-α to the post-γ positions, i.e. it is polyclonal. Thus when we find an increase with a narrow electrophoretic mobility and confirm that it contains only one class of heavy chain and one type of light chain, we believe this represents the product of a single clone of cells, i.e. it is monoclonal.

There are a few rare exceptions to these findings. Polymerization or cryoprecipitation can result in a paraprotein 'trailing' on electrophoresis, although immunochemical typing shows only one class of heavy chain and/or one class of light chain. These are sometimes called broad-banded myeloma proteins, but I must stress that when cellulose acetate is used they are exceptionally rare, and we have only found 2 such examples among 600 paraproteins and 10,000 other sera, both of which showed some banding. Sometimes more than one narrow band is found due to polymers or the presence of more than one clone, as in some 2% of cases of myelomatosis (*see* Fig 1).

In normal antibody-forming cells and well-differentiated plasmacytoma cells immunoglobulin synthesis is so well balanced that in radio-isotope labelling experiments less than 1% of the radioactivity escapes from the cell as free light chains (Askonas & Williamson 1967). In myelomatosis, &c., cells can become unbalanced and free light chains can appear as Bence-Jones proteins: this imbalance can go further so that no heavy chains appear and only Bence-Jones proteins are detectable. Furthermore the neoplastic plasma cells can produce *less* protein than normal. This loss of normal function parallels increasing malignancy, and a few myelomata fail to release any immunoglobulin at all.
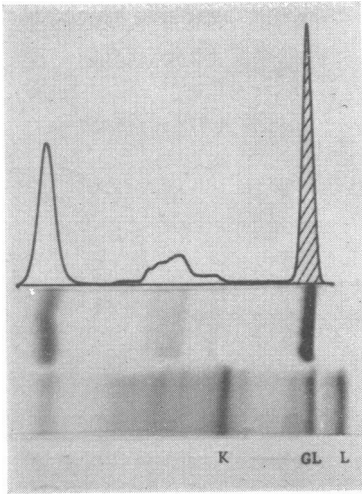
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Fig 1 *Malignant paraproteinæmia. Electrophoresis on cellulose acetate of serum* (above) *and concentrated urine* (below) *reveals:* (1) *Bence-Jones proteinuria; in this case two monoclonal bands are seen, one of each type.* (2) *Loss of normal* γ-*globulin.* (3) *A high level of M-protein*

Dr A Corbett has reviewed 402 patients in whom we found Bence-Jones proteins and on follow up of these reticuloendothelial malignancy of all types, but mostly myelomatosis, has been proven in 400. Bence-Jones proteins are monoclonal light chains and are evidence of dedifferentiation. This biochemical dedifferentiation is associated with clinicopathological worsening; such myelomata present earlier, grow faster, have more complications and the patients die sooner. In some cases only fragments of heavy chains are found.

Here I use the term paraprotein only where a narrow electrophoretic mobility has been demonstrated and immunochemically confirmed as immunoglobulin or fragments thereof with heavy and/or light chains of one type only.

The incidence of paraproteins in a natural population of 7,000 has been recorded by Hällén (1966). Over 50 years of age, 1% had serum paraproteins and over 70 years, 3%. In a hospital population of 7,000 I found an identical incidence. We both believe these paraproteins mean that a monoclone of reticuloendothelial cells has grown within our subjects. The question for the clinician is whether such monoclones will grow on and invade other tissues like malignant tumours, whether they will strike an equilibrium with their neighbours like benign tumours or even behave like normal antibody cells (as I believe is the case for the rare transient paraproteins) which appear,

increase and then switch off. Hällén thought that most of the paraproteins in his natural population were of benign significance. After following up our hospital patients for three years I found two-thirds represented reticuloendothelial malignancy (Hobbs 1967). Axelsson & Hällén (1968) recently reported their 2½ year follow-up study and consider the vast majority outside hospital to be benign.

Returning to the patients we see attending hospital, certain features were found of value in predicting the likelihood of malignant behaviour (Hobbs 1966).

In order of importance these were: (1) Bence-Jones proteinuria. (2) Loss of normal immunoglobulins. (3) A high level of paraprotein (*see* Fig 1). (4) A tendency for the level to increase relentlessly.

In contrast, where (1) no Bence-Jones proteinuria could be found, (2) no loss of normal immunoglobulins could be detected, (3) a low level of paraproteins was seen (*see* Fig 2), and (4) this did not increase significantly on follow up – then a benign outcome was probable.

The diagnoses achieved in patients with paraproteinæmia are shown in Table 1, and I am mainly concerned with the 74% who had malignant neoplasia of reticuloendothelial origin. Most common was myelomatosis, then Waldenström's macroglobulinæmia, primary soft-tissue plasmacytoma, lymphosarcoma, plasma cell leukæmia, chronic lymphatic leukæmia and even typical giant follicular lymphoma. Some histology has been very difficult to classify and has been called atypical Hodgkin's disease and reticulosarcoma and some clinical patterns closely resemble myelosclerosis and allied syndromes. Thus in all these cases the malignancy was of cell lines derived from the reticuloendothelial system.

Over the years it has been believed there is an association between paraproteins and other forms of malignancy. However, Osserman (1958) found paraproteins in only 7 of 2,000 patients with various cancers, Seitanidis (1966) found them in 3 of 146 patients and we have seen paraproteins in only 2% of 400 patients with cancer. Since the commonest age group in all our series was 60–70 years, the natural incidence of paraprotein could be expected to be about 2%. Thus the association seems purely fortuitous – there is not even a slight excess of paraproteins in cancer subjects. Furthermore, I know of no convincing evidence of a paraprotein (as defined above) which disappeared or was reduced after removal of a

Table 1
Diagnoses achieved in 693 patients with paraproteins

Clinicopathological picture	Paraprotein	No. of cases
I. *Malignant reticuloendothelial neoplasia:*		
Myelomatosis (includes 5 patients with plasma cell leukæmia)	Only γG 20%	
	γG + BJ 33%	
	Only γA 6%	
	γA + BJ 16%	
	Only BJ 20%	
	γD + BJ 1·5%	420
	γM + BJ 0·5%	
	None 1%	
	Biclonal 2%	
	(or more)	
Waldenström's macroglobulinæmia	(19s γM)	32
Soft-tissue plasmacytoma	Only BJ 10	
	Only γG 3	
	γG + BJ 3	
	0·5mol γG 2	24
	γM + BJ 1	
	γD + BJ 1	
	Not detected 4	
Lymphosarcoma	19s γM 16	
	7s γM 1	18
	Only BJ 1	
Reticulosarcoma ●	BJ 3	
	γG 1	
	γA 1	6
	γM 1	
Chronic lymphatic leukæmia	γG 2	5
	BJ 3	
Myelosclerosis	γA 1	2
	γG 1	
Giant follicular lymphoma	BJ	1
Arabian lymphoma	Polymer α-chain	1
II. *Monoclonal antibody:*		
Primary cold agglutinin syndrome (benign growth 37, terminating as lymphoma 4, presenting as lymphoma 4 ■)	γMK	45
Lichen myxœdematosus	γGL	4
Transient paraproteins	γG	5
III. *Benign:*		
Mainly static for over three years	Only γG or γA or γM or γD	112
IV. *Uncertain*		18

● includes 5 atypical Hodgkin's disease
■ incidental cold agglutinin activity in the γM
BJ = Bence-Jones

carcinoma, nor of any fluorescent antibody study showing carcinoma cells with only the paraprotein type of immunoglobulin. In 4 patients with cancer and identified paraproteins the cancer cells have been grown in tissue culture and tested for production of immunoglobulin. None has been detected.

In the individual patient it is unlikely that the class of paraprotein in itself will indicate the clinical diagnosis. It is true γG, γA and Bence-

Jones proteins alone are most commonly associated with myelomatosis, but all are recorded with lymphoma. Similarly, while γM most often is found with Waldenström's clinical picture, in other cases there is frank lymphosarcoma and, in a few, undoubted myelomatosis. The primary cold agglutinin syndrome is due to γM paraprotein showing an affinity for the I antigen of red cells. Most often the cells producing the cold agglutinin themselves behave in a benign manner, but about 10% of Professor J V Dacie's 40 patients have manifested frank invasion, terminating as malignant lymphomata. Thus, this syndrome shows overlap between the malignant and benign clones. It is also one of the few where the paraprotein is nearly always γMK and can be shown to have specific antibody activity. There is another rare condition, lichen myxœdematosus, where a paraprotein is found (James *et al.* 1967). It is always of very slow mobility, γGL, of low level not obviously rising on follow up, without Bence-Jones proteinuria and no loss of normal immunoglobulins (*see* Fig 2). Since γG-globulin can be shown in the skin lesions, this paraprotein too may have specific antibody activity causing its own disease picture.

Coming to the rarer paraproteins, γD and γE have been found with myelomatosis. A dimer of part of the Fc-fragment of the γ-chain has been
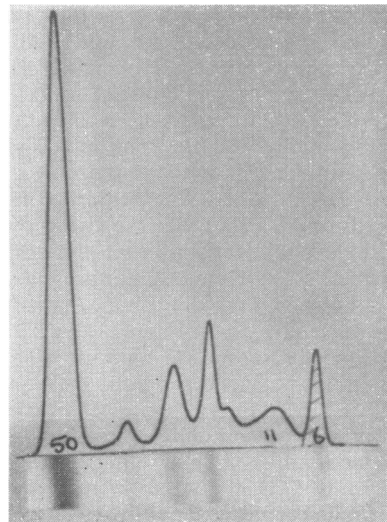

Fig 2 *Benign paraproteinæmia. Electrophoresis on cellulose acetate of serum and concentrated urine reveals:* (1) *No Bence-Jones proteinuria.* (2) *No loss of normal γ-globulin.* (3) *A low level of M-protein. In this case the M-protein is of post-γmobility, is type γGL and is typically, but not exclusively, found in lichen myxœdematosus*

found in the heavy-chain disease of Franklin, which seems largely to involve peripheral lymph-nodes (Ellman & Bloch 1968). Seligmann *et al.* (1968) reported α-chain with Arabian lymphoma. This occurs in young people of Middle-East origin and involves the whole of the small gut. We have also seen this in a patient of Dr F Avery Jones's. The protein is most unusual, being a highly polymerized broad band of α-chains, without light chains. The half-molecule γGK has been identified in a patient with soft-tissue plasmacytoma (Hobbs & Jacobs 1969). We have also found half-light chains only (mol. wt. 10,000) in 2 patients with myelomatosis. To date immunoglobulin fragments have been found only with reticuloendothelial malignancy and seem to provide chemical evidence of dedifferentiation.

Finally, I want to consider the rate of production of malignant paraproteins. In Potter's mice it was shown that the serum level of paraprotein was directly proportional to the weight of soft-tissue plasmacytoma. Using an ascitic form of tumour kindly provided by Dr B A Askonas, my research assistant Mr O Fakhri has been using radioisotope dilution and counting the total number of tumour cells within the ascitic fluid. Plotted against the serum level of paraprotein a simple linear relationship was found confirming that the serum level reflects the growth of a monoclone.

In some patients with myelomatosis the rate of rise of serum level has been followed (Hobbs 1969). Growth appears to be exponential, the doubling time varying from 3·5 to 24 months for γG-paraproteins. On average it requires the daily production of 14 g γG in a 70 kg subject to maintain the serum level found at the clinical presentation of myelomatosis. This requires 1 kg of myeloma cells or $4·6 \times 10^{11}$ cells. We also know from Dr F G J Hayhoe that the average myeloma marrow contains 33% myeloma cells at presentation and from the known values for bone marrow this would be about 1 kg of myeloma cells. The earliest detection of a serum γG paraprotein at 0·2 g/100 ml would be at 43 g tumour or 9,000 million cells (*see* Fig 3). In the mouse Mr Fakhri has found that the paraprotein is first seen in serum when there are 3 million cells, and as a mouse's weight is 1/3,000 that of a man this experimental finding confirms the above estimates.

In man this exponential growth has actually been observed (*see* the solid line in Fig 3) and on average it takes five years from earliest chance detection to clinical proof. If the dotted line extrapolation is also true, as in the mouse, then it takes 33 years for a single neoplastic cell to
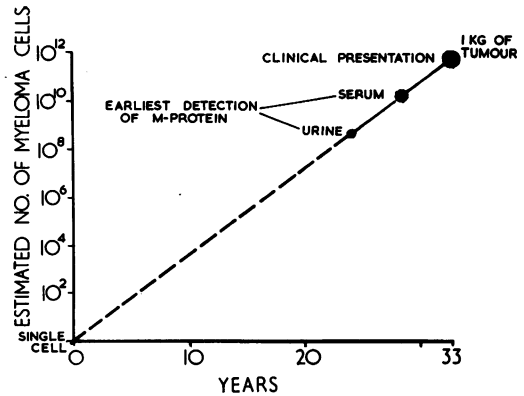

Fig 3 *The natural history of γG-myelomatosis. The solid line shows the actually observed logarithmic increase in serum M-protein level, taking 5 years from earliest chance detection to the average amount at clinical presentation, which the broken line extrapolation suggests takes 33 years from a single cell. Bence-Jones proteinuria might be detected 10 years before clinical evidence*

grow to a degree presenting as clinical myelomatosis.

For γA the average estimate is 21 years and for the dedifferentiated myelomata producing only Bence-Jones proteins, the average is 11 years. Even here our fastest doubling time is one month. This is much slower than for normal antibody formation, which doubles in days.

To sum up, suspected paraproteins seen on electrophoresis should be confirmed immunochemically. Electrophoresis of concentrated urine and measurement of normal immunoglobulin are valuable investigations. If the diagnosis is in doubt follow up is needed for up to at least 5 and sometimes 10 years before a benign prognosis can be assumed. Because of the usually slow growth rates we follow up at yearly intervals unless there are special circumstances.

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