Protein analyses in myelomatosis

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The criteria for the admission of patients to the Medical Research Council's (MRC) myeloma trials are the finding of abnormal plasma cells in bone marrow films or sections and in addition either of characteristic skeletal lesions demonstrated radiologically or of characteristic protein changes in the serum or urine. Ninety per cent of the patients in the first myeloma trial satisfied all three criteria (Medical Research Council, 1971).

Before the introduction of alkylating agents in the treatment of myelomatosis the median survival of patients with this disease was 17 months after the onset of symptoms and seven months after the start of treatment (Farhangi and Osseman, 1973). The survival of patients is now much longer and this is due partly to the introduction of alkylating agents such as melphalan and cyclophosphamide and partly to improved techniques of biochemical monitoring of the tumour. The subject of this article is the monitoring of immunocytomas by biochemical analyses.

The Detection of Paraproteins

Plasma cells in the marrow and lymphoid tissue normally make complete immunoglobulins. The two heavy polypeptide chains are synthesized on large polyribosomes in about two and a half minutes, and the two light polypeptide chains are synthesized on smaller polyribosomes in about one minute (Askonas and Williamson, 1967). The four polypeptide chains are then assembled and carbohydrate is added in the Golgi apparatus. The production of heavy and light chains is well balanced, there being normally a very small excess of light chains synthesized. About 2 to 3 mg of polyclonal free light chain is excreted into the urine per day.

Differing chemical properties of the heavy chains allow immunoglobulins to be divided into distinct classes designated G, A, D, E, and M. Light chains belong to one of two types, κ and λ , which are common to all classes. Immunoglobulins in classes G, A, D, and E are, in normal adult serum, monomers of the basic four-chain unit, while those in class M are pentamers, and each immunoglobulin mole-

cule contains light chains of a single type only. In a normal mixture of immunoglobulins 70% of the molecules are type κ and 30% are type λ .

Although normal immunoglobulins in any class are heterogeneous and form a broad region on cellulose acetate electrophoresis, according to Burnett's theory each plasma cell synthesizes immunoglobulins of one molecular type only (Marchalonis, 1968). Immunocytomas are tumours derived from a single cell (Waldenström, 1962) and the immunoglobulin products of all the cells in a given tumour, whether they are complete molecules or only fragments, are therefore identical. Such homogeneous products appear as discrete bands when stained after cellulose acetate electrophoresis and are termed 'paraproteins'. Some immunocytomas synthesize whole immunoglobulin molecules only but others synthesize immunoglobulin fragments either in addition to the whole molecules or as their sole product, and the significance of this is discussed later. By far the commonest fragment produced is intact light chain (molecular weight about 22 000) which is excreted through the kidney and is commonly present in the urine as a dimer. These light chains are the Bence-Jones proteins. It is very much rarer for heavy chains to be made in excess of light chains (Seligmann, 1972) and, when present, they are usually incomplete polypeptide chains.

The investigation of paraproteins can be of value in the diagnosis of the underlying immunocytoma and for monitoring its progress. Serum, ascitic fluid, and cerebrospinal fluid have all been analysed for the presence of whole-molecular paraproteins and urine has been studied for small molecular weight fragments such as Bence-Jones proteins which can be excreted through the kidney. If paraprotein studies in body fluids are inconclusive, marrow tumour cells may be investigated (Hobbs, 1967) although this is rarely necessary.

Paraproteins are usually investigated by cellulose acetate electrophoresis and immunoelectrophoresis of serum and urine. Electrophoresis may be used to quantitate the amount of paraprotein present if the cellulose acetate strip is stained on completion of a

run and the proportion of dye bound to the paraprotein related to the total protein value of the sample. The techniques involved have recently been reviewed by Kohn (1973). Before electrophoresis, urine may be concentrated up to 300 times by vacuum extraction through a collodion membrane (McLaughlin, 1972) and with this technique the limit of detection of Bence-Jones protein can be reduced to about 6 mg/l. Serum paraproteins may be detected by electrophoresis down to a level of about 2 g/l.

After detection of a discrete band on electrophoresis, the presence of a paraprotein may be confirmed by abnormal reactions on immunoelectrophoresis, such as bowing or splitting of the arcs; Bence-Jones proteins diffuse rapidly because of their small size and characteristically form precipitin arcs within two hours of adding antiserum to the plate.

In addition to confirming the presence of a paraprotein, immunoelectrophoresis also identifies the class of the abnormal immunoglobulin. In the first MRC myeloma trial, of 258 patients 55% had an IgG paraprotein, 26% had an IgA paraprotein and 19% Bence-Jones proteins only. Eight patients had two paraproteins and five others had an IgD paraprotein (Medical Research Council, 1973).

The detection of Bence-Jones protein by chemical methods is not reliable (Hobbs, 1966). The Bradshaw

ring test is perhaps the best, although even this may fail to detect about 1 in 20 Bence-Jones proteinurias.

Significance of the Presence of Paraproteins

The incidence of paraproteins in the serum of a natural population is as high as 1% over the age of 50 and 3% over the age of 70 (Hällén, 1966). Most paraproteinaemias in populations outside hospital are probably benign (Axelsson and Hällén, 1968), but this is not so for hospital patients. The diagnoses reached in 691 inpatients found to have serum paraproteins (Hobbs, 1969a) are shown in table I. About three-quarters of the patients had a wide range of malignant immunocytomas, myeloma being the most common, and about one quarter had benign conditions.

Paraproteins have rarely been reported in association with malignancies other than immunocytomas and a study of 2000 patients with various cancers (Osserman, 1958) revealed only seven paraproteins, no more than the incidence for an age-matched normal population.

When a paraprotein has been found and identified, the laboratory results can be used to assess whether the underlying lesion is likely to be malignant or benign. Table II shows the incidence of some biochemical features in 517 patients with malignant

Diagnosis	No. of Patients	Incidence (%)
1 Malignant immunocytomas (515 cases)		74
Myelomatosis	420	
Waldenström's macroglobulinaemia	32	
Soft tissue plasmacytoma	20	
Lymphosarcoma	26	
Reticulosarcoma	6	
Chronic lymphatic leukaemia	5	
α-Chain disease	3	
Atypical myeloid sclerosis	2	
Giant follicular lymphoma	1	
2 Benign immunocytomas (158 cases)		23
No associated pathology	112	
Monoclonal antibodies:	46	
Primary cold agglutinins	37	
Lichen myxoedematosis	4	
Transient paraproteins	5	
3 Uncertain	18	3

Table I Diagnoses in 691 inpatients with detectable serum paraproteins

Immunoglobulin Disorder	Incidence (%)	
	Malignant (517 Patients)	Benign (112 Patients)
Immunoglobulin fragments present	84	0
Immune paresis present	98	10
Paraprotein level above 10 g/l serum	92	15
Increase in paraprotein level with time	99	1

Table II Incidence of certain immunological disorders in 517 patients with malignant immunocytomas and 112 with benign immunocytomas

immunocytomas diagnosed by biopsy and in 112 patients with benign immunocytomas who had been followed for at least five years. Other findings which contribute to the diagnosis include, of course, marrow biopsies and radiography, especially of the axial skeleton. A low haemoglobin, low serum albumin and high serum urea are further pointers to malignancy.

The finding of paraproteins in patients with malignant immunocytomas is, however, not invariable and 1% of patients with myelomatosis do not synthesize sufficient paraproteins for them to be detected in the serum by electrophoretic techniques (Hobbs, 1975).

Assessing Immunocytoma Mass

Apart from being diagnostically helpful, paraprotein studies may also be of value in assessing the size of the underlying immunocytoma.

It was found from studies on experimental plasmacytomata in mice that the weight of tumour was proportional to the turnover of the associated paraprotein (Nathans, Fahey, and Potter, 1958). Later it was found that tumour weight was also related to the serum level of paraprotein (Osserman, Rifkind, Takatsuki, and Lawlor, 1964), and Fakhri and Hobbs (1970), using ^{51}Cr -labelled red cells to assess peritoneal fluid volume, showed a direct relationship between the number of plasmacytoma X5563 cells in the ascitic fluid of C3H mice and the serum paraprotein level (fig 1).

Studies *in vitro* on marrow have shown that the amount of paraprotein synthesized by human myeloma cells is relatively constant at about 0.5×10^{-11} to 3.4×10^{-11} g/myeloma cell/day both for different cultures and also for the same culture over a period of time (Salmon and Smith, 1970). The quantity of paraprotein synthesized in a given patient therefore varies with the tumour mass (Salmon and Smith, 1970; Salmon, McIntyre, and Ogawa, 1971), but the relationship with serum concentration is not a simple one for all immunoglobulin classes, since the half-lives *in vivo* of some immunoglobulins vary as a function of serum concentration (Schultze and Heremans, 1966). Although the fraction of the intravascular mass of IgG₃, IgA, and IgM which is catabolized daily probably does not vary with changing serum concentrations, it does vary for IgG₁, IgG₂, and IgG₄ (Salmon, 1973).

Estimations of serum paraprotein levels do, however, allow assessments of tumour size, except in the 1% of cases of myelomatosis in which paraprotein synthesis is inadequate for detection by electrophoresis of the serum.

The average serum paraprotein levels in patients with myelomatosis at the time of clinical presentation are shown in table III. It can be calculated from

Paraprotein	Level in Serum or Urine
IgG (subclasses 1, 2, 4)	43 g/l in serum
IgG (subclass 3)	35 g/l in serum
IgA	28 g/l in serum
IgD	14 g/l in serum
Bence Jones only	5.4 g/24 hours in urine

Table III Average serum paraprotein levels in patients with myelomatosis at clinical presentation

the turnover rate *in vivo* that for IgA the serum level of 28 g/l reflects a tumour mass of about 4.6×10^{11} cells, approximately 1 kg of tumour. Similar calculations for IgG and IgD, allowing for differences in their turnover rates at different serum levels, indicate that for these proteins also, the average serum levels found on clinical presentation reflect a tumour mass of about 1 kg. A similar value has also been reported by other authors for various immunoglobulin classes including an IgE paraprotein (Salmon *et al*, 1971).

Examination of bone marrows from 230 patients with myelomatosis at the time of clinical presentation revealed on average a 33% replacement with tumour cells (Hobbs, 1971). Since it has been estimated that the average weight of bone marrow in a 70-kg patient is 3.2 kg (Mechanik, 1926) these findings too suggest the presence of about a kilogram of myeloma.

Fig 1 Serum paraprotein levels in C3H mice related to the number of plasmacytoma X5563 cells present in the ascitic fluid.

Kinetics of Tumour Growth

The way in which immunocytomas grow is the subject of some debate. Using a 'sandwich' radio-immunoassay technique to estimate nanogram quantities of paraproteins secreted by fresh myeloma cells *in vitro* (Salmon, Mackey, and Fudenberg, 1969) 70 myeloma patients were studied over a period of two to four years. The findings suggest that, while tumour growth is initially exponential, growth rate slows as the tumour enlarges and, although the doubling time of the tumour on presentation may be four to six months, it initially could be very much shorter, perhaps one to three days (Sullivan and Salmon, 1972). It has been argued that the growth of all animal tumours may be better described by a Gompertzian growth curve¹ (Laird, 1964) than by a first-order kinetic model and this would imply that myelomas take only one to two years to develop from a single cell to a tumour which presents clinically. Support for the concept of very rapidly enlarging immunocytomas is given by the appearance of bands on electrophoresis only a few weeks after antigenic challenge during monoclonal responses to severe infection such as hepatitis or to bone marrow transplantation (Salmon, 1973), and by the generation time of myeloma cells *in vivo* as determined with tritiated thymidine (Killman, Cronkite, Fleidner, and Bond, 1962).

The growth of plasmacytomas in mice, however, has been shown to be exponential to a tumour size of 10^8 cells (Fakhri and Hobbs, 1970) and studies of nearly 1000 MRC trial patients have enabled observations to be made on 94 untreated patients with progressive myelomatosis which also support the idea of simple exponential growth (Hobbs, 1967).

The median tumour doubling time for IgA myelomatosis at first presentation is 6.3 months (Hobbs, 1969b) and for IgG myelomatosis about six months also (Salmon and Smith, 1970). Extrapolation, assuming simple exponential growth at this rate, suggests that 21 years is required for an IgA myeloma to develop from one cell to a kilogram of tumour (Hobbs, 1971) and about 33 years for an IgG myeloma (Hobbs, 1969c).

There is much evidence to support the existence of a very long preclinical period in myelomatosis: (1) recurrence of disease has been reported as long as 17 years after removal of a solitary plasmacytoma (Wiltshaw, 1969); (2) accidental detection of paraproteins has been reported 15 to 24 years before the clinical emergence of myelomatosis (Nørgaard, 1971); (3) no patients under the age of 29 had been reported with IgG or IgA myeloma (Hobbs, 1969b); (4) six patients in the MRC myeloma trial were found to

¹Progressive slowing of the rate of growth.

have a paraprotein eight years before any tumour was clinically evident; (5) one patient is reported to have developed an IgG myeloma with paraprotein antibody activity against horse α_2 -macroglobulin 33 years after an injection of horse serum (Seligmann, Sassy, and Chevalier, 1973).

A myeloma in most of the above patients could not have evolved in the length of time suggested by Gompertzian growth kinetics. Because of the nature of the tumour, it may be that myeloma growth kinetics differ from those of more discrete tumours. Whether tumour growth is Gompertzian or exponential throughout the natural history of the disease, serum paraprotein levels do reflect the mass of the underlying tumour and may therefore be used to monitor changes in tumour mass over a period of time.

Monitoring Tumour Responses to Therapy

Without treatment, less than 50% of patients with myelomatosis survive one year from clinical presentation (Feinleib and MacMahon, 1960; Innes and Newall, 1961). In patients surviving six months to one year after clinical presentation there would be a further one to two doublings in tumour mass to about $1-2 \times 10^{12}$ cells, about 3 kg of tumour, causing bone marrow failure. If areas other than normal red marrow are invaded, larger masses of tumour may be tolerated and higher levels of serum paraproteins observed.

The introduction of alkylating agents has greatly extended the time of survival of patients with myeloma by causing reductions in tumour mass.

Many criteria have been used to assess the responses of tumours to chemotherapy. It has been suggested that a good response would be a 10 to 100-fold reduction in tumour mass (Salmon, 1973) but criteria proposed by the Chronic Leukaemia Taskforce of the National Cancer Institute (cited by Snapper and Kahn, 1971) included a 50% reduction in both serum paraprotein concentration and Bence-Jones proteinuria compared with initial levels. By these latter criteria about 82% of patients in the first MRC myeloma trial would be considered to have responded to chemotherapy.

Observations on MRC myeloma trial patients over nine years indicate that paraprotein levels reflect the response to treatment in 98% of patients if both serum and urine are investigated (Hobbs, 1974). In 2% of patients, however, a tumour may continue to enlarge with no corresponding rise in serum paraprotein levels.

There is a variable rate of fall of serum paraprotein levels in patients responding to chemotherapy, not explained by differences in the *in-vivo* half-lives of the

Fig 2 Changes in serum IgG₁ paraprotein levels in four representative patients with similar initial levels of paraprotein during treatment of myelomatosis with cyclophosphamide or melphalan. Slow reduction is when the paraprotein level falls to 50% of the initial level but takes more than three months, and rapid reduction is when the paraprotein level falls to 50% of the initial level within three months.

various immunoglobulin classes and subclasses (fig 2). The effects of chemotherapy on serum paraprotein levels were studied in 167 patients. One hundred and seven patients (64%) showed a slow response (a reduction of paraprotein level by 50% but not within three months), 30 patients (18%) showed a fast response (a reduction of paraprotein level by 50% within three months), 25 patients (15%) showed little change, and five patients (3%) showed an increasing paraprotein level (Hobbs, 1969c). The responses to therapy of myeloma patients with Bence-Jones proteinuria only show a similar pattern.

Whatever the rate of response to chemotherapy, reduction in tumour size has been shown to fit Gompertzian kinetics (Sullivan and Salmon, 1972). Their explanation is that as the tumour size decreases so the 'proliferating compartment' of the tumour increases. Eventually the tumour size reaches a plateau where there is a balance between an increased tumour cell death due to chemotherapy and an increased tumour cell birth due to an expanded proliferating

compartment. Such retarded regression would be expected with drugs which produce a constant fractional kill of tumour cells. If the 'plateau' amount of tumour is such that the amount of paraprotein synthesized is below the level of detection by electrophoresis, monitoring of this phase of the disease becomes impossible by usual methods and increases in tumour size will only be detected when the paraprotein again becomes visible on the electrophoretic strip. Another possibility is that chemotherapy selects those subclones of the tumour which are more resistant to the cytotoxic agents.

Factors of Prognostic Significance

The investigation of patients both at clinical presentation and subsequently has enabled the prognostic evaluation of many factors. Investigations of diagnostic value tested for prognostic value in patients given continuous cytotoxic treatment in the first MRC myeloma trial, and found to be of no prognostic significance, are shown in table IV (Peto, 1971). It is interesting that the serum paraprotein level at presentation is of no prognostic value in patients on chemotherapy.

Parameters at diagnosis which have been found to be of prognostic value are listed in table V; each is of value independently of the others.

High serum urea and heavy non-Bence-Jones proteinuria independently indicate renal damage, and a low haemoglobin may indicate preexisting bone marrow suppression limiting effective cytotoxic chemotherapy.

The presence of Bence-Jones proteinuria reflects biochemical dedifferentiation and may indicate the presence of a more dedifferentiated and faster growing tumour (Hobbs, 1969c). The finding of any immunoglobulin fragment in the sera of patients with these tumours would have similar significance (Hobbs, 1971). The production of Bence-Jones protein alone, which may reflect an even greater degree of tumour dedifferentiation, is associated with a very poor prognosis (Medical Research Council, 1973).

Whether the Bence-Jones protein present is type κ or λ may also be important. The median survival of 22 patients in the first MRC myeloma trial with type-

Radiology	Serum	Blood
Lytic bone lesions	Calcium	Leucocyte count
Wedging of vertebrae	Alkaline phosphatase	Platelet count
Collapse of vertebrae	Uric acid	
Osteoporosis	Paraprotein level	

Table IV Factors at diagnosis of no prognostic significance in patients receiving continuous cytotoxic treatment

Investigation		Death Rate Relative to an Overall Value of 1.0
Serum albumin (g/l)	Above 30	0.7
	20-29	1.1
	Below 19	1.8
Serum urea (mmol/l)	Below 6.7	0.6
	6.7-13.1	1.1
	Above 13.1	3.2
Non-Bence-Jones proteinuria (g/l)	Below 0.4	0.8
	Above 0.4	1.6
Bence-Jones proteinuria (g/l)	Below 0.1	0.7
	Above 2.0	3.4 ¹
Haemoglobin (g/dl)	Above 10.0	0.5
	7.5-9.9	1.1
	Below 7.5	2.0

Table V Parameters at diagnosis of prognostic significance in patients receiving continuous cytotoxic treatment

This high value is associated with an increased incidence of uraemia

Bence-Jones-only myeloma was five months, whereas the median survival of 31 patients with type κ Bence-Jones-only myeloma was only two months.

Serum albumin levels on clinical presentation are also of prognostic value and vary independently of other factors such as proteinuria or paraprotein level on presentation. The survival of patients in the first MRC myeloma trial related to the serum albumin concentrations on admission to the trial is shown in figure 3.

The reasons for the correlation of original serum albumin levels with prognosis are not fully understood; low albumin values have been reported in a wide range of both benign and malignant diseases (Zilva and Pannall, 1971). It has been shown, however, that in tissue culture normal cells pinocytose albumin in small regular droplets, whereas malignant cells engulf albumin in larger, irregular droplets (Cohen, Beiser, and Hsu, 1961). It may be suggested therefore that an originally low serum albumin level, not due either to protein loss from the body or hepatic disease, could indicate a high consumption of albumin by the tumour. Although our preliminary findings tend to support this explanation, no conclusive studies on albumin turnover in patients with myelomatosis have yet been reported.

Apart from features present at clinical presentation, the rate of response of the tumour to chemotherapy, as previously defined, has also correlated with subsequent prognosis (Hobbs, 1969c). The survival of 167 patients in the first MRC myeloma trial according to the rate of response to chemotherapy is shown in figure 4. It can be seen that the survival of those who responded slowly was better than those whose response was fast. It may be that

Fig 3 Survival of patients in the first MRC myeloma trial related to their serum albumin concentration on admission to the trial.

Fig 4 Survival of 167 patients in the first MRC myeloma trial related to their response to chemotherapy, whether fast (paraprotein level falling to half initial level within three months, 30 patients) slow (paraprotein level falling to half initial level but not within three months, 107 patients) or insignificant (30 patients).

those tumours which respond rapidly to chemotherapy are those with the highest proportion of mitotic cells and that a slow response reflects a low frequency of mitosis.

Results of Therapy

Of the 258 patients in the first MRC myeloma trial 45% were alive two years after diagnosis and 11% five years after diagnosis. Survival rates as high as 40% of patients five years after diagnosis with an average survival of 55 months have been claimed for other series (Farhangi and Osserman, 1973).

During studies of patients admitted to the first MRC myeloma trial (1964 to 1968) serum paraproteins became undetectable in 21 patients of whom nine are still alive and well. This group forms a large proportion of the 17 patients still surviving from this trial.

Despite the introduction of chemotherapy, therefore, perhaps half the patients with myelomatosis die within two years of diagnosis. There are many causes of death and in one series of 145 fatal cases of myelomatosis (Hobbs, 1971) the cause of death was not related to the myeloma in seven patients, indirectly related to the myeloma in 11 patients, due to a failure to respond to chemotherapy and relentless progression of the tumour in 40 patients and due to an escape from chemotherapy after an initial response to therapy in 87 patients.

This last group of patients accounted for 60% of the deaths in this series, and the ways in which they escaped from therapy after initially responding are shown in table VI. Simple escapes are those

their very actions, generate mutations (Hobbs, 1971).

Fingerprint studies both of original paraproteins and of different paraproteins which have appeared subsequently during treatment may show single peptide changes due, perhaps, to single amino acid changes (Hobbs, 1975). Such changes could be explained by one-point alterations to single DNA codons. The escape from treatment may also be due to the development of another malignancy such as a reticulosarcoma or a monocytic leukaemia. The risk of another such malignancy developing during treatment with cytotoxic agents increases after five years of chemotherapy (Osserman, 1969). Where a diagnosis of myeloma has been clearly established such a risk is worth taking. Where it is possible that the underlying lesion is a benign immunocytoma, however, it is probably best to wait until the diagnosis of malignancy becomes more certain before commencing cytotoxic therapy.

Current Trends in Techniques of Monitoring

Serum paraprotein and urine Bence-Jones protein levels have so far been detected and quantitated routinely only by electrophoretic and dye-binding techniques within the relatively insensitive limits of detection already mentioned.

We have recently developed a rocket immunoselection technique for the detection of free light chains in unconcentrated urine down to a level of 3 to 5 mg/l. The technique is similar in principle to that recently described for the detection of heavy chain fragments (Gale, Versey, and Hobbs, 1974). The sample is run through an agarose zone containing anti-heavy-chain antisera which precipitates all the intact immunoglobulins. Free light chains continue to migrate anodally towards another agarose zone containing anti-light-chain antisera against which precipitin 'rockets' are formed. Figure 5 shows the reactions produced by normal urines and by doubling dilutions of specimens of urine from two patients with Bence-Jones proteinuria at initial concentrations of 0.5 g/l. The normal urines produce no reaction and the difference in peak heights for the neat samples from the two patients with Bence-Jones proteinuria reflects the differing reactivity of the two monoclonal proteins with the same antiserum. Although positive results would have to be confirmed as representing Bence-Jones protein by the demonstration of a discrete band on electrophoresis of concentrated urine, negative results eliminate the possible presence of Bence-Jones protein. This technique could therefore be useful as a very sensitive test for large numbers of urines and would also be useful for follow-up studies on patients known to have Bence-Jones proteinuria.

Modes of Escape	Incidence (%)
Simple escape	55
Growth rate escapes	(42)
(a) Same paraprotein during relapse	2
(b) Different paraprotein during relapse	5
(c) More Bence-Jones during relapse	35
Non-myeloma escapes	(3)
(a) Reticulosarcoma	2
(b) Monocytic leukaemia	1

Table VI Escapes from therapy in patients with myelomatosis

where the paraprotein level rises at a rate identical with the rate of rise before the onset of therapy whilst growth rate escapes are those where the paraprotein level rises faster than before, reflecting an increased tumour growth rate.

There are a number of ways in which increased tumour growth rate may be detected: (1) there may be an increased rate of appearance of the same serum paraprotein that was present before the onset of chemotherapy; (2) there may be an increased rate of synthesis of Bence-Jones protein only, by the tumour; (3) the paraprotein which increases during treatment may be different from that present before the onset of chemotherapy.

All the growth rate escapes in the MRC studies have occurred after at least six months of cytotoxic therapy and it may be that the treatment has either selected or generated a faster growing element from the original clone. The latter is the more likely as our current cytotoxic agents acting on DNA may, by

Fig 5 Immunoselection plate showing precipitin arc formations in the middle zone (B) between anti-light-chain antisera (Dakopatts) initially present in the anodal zone A and doubling dilutions of specimens of urine from two patients with Bence-Jones proteinuria (wells 5-9 and 10-14 respectively) at initial concentrations of 0.5 g/l each. The specimen origin wells (o) are in zone C, nearest the cathode, which contains anti-heavy-chain antisera. No reaction is seen against normal urines placed in the four origin wells on the left.

To gain more information concerning the kinetics of small masses of tumour-producing amounts of paraprotein below our current techniques of detection and to detect any escape from chemotherapy earlier than is possible with the currently used routine methods, we also need more sensitive techniques for the detection of serum paraproteins.

Immunochemical techniques for the estimation of serum paraproteins, such as radial immunodiffusion (Fahey and McKelvey, 1965), have the disadvantage that a polyclonal antiserum will react differently with different monoclonal proteins because the antibodies with high affinity for these particular proteins are present in different concentrations. The amount of specific antibody present in a polyclonal antiserum may be so small relative to the amount of antigen as to produce a false increase in the diameter of the precipitin ring compared with standards which consist of a mixture of immunoglobulins reacting with other antibodies in the antiserum.

It is possible to overcome the problems of radial immunodiffusion by estimating the paraprotein in an environment of antibody excess by the light-scattering effect of antibody-antigen complexes formed in solution (as in the automated immuno-

precipitin system) but reaction between antisera and normal immunoglobulins obscures the investigation of low levels of paraproteins. Another approach is to separate the paraprotein from the patient's serum, raise a specific antiserum in animals and use it to follow that particular patient's paraprotein level. This technique is to be one of the studies in the third MRC myeloma trial due to start in the near future and should allow much earlier detection of escape from chemotherapy by enabling quantitation of paraproteins below the limit of detection by electrophoresis.

Conclusions

The current fundamental concepts concerning paraproteins (Hobbs, 1971) are therefore: (1) that they are the product of a monoclonal of cells; (2) that the serum level of paraprotein reflects the mass of immunocytoma present, both during the natural history of these malignant disorders and also during reduction of tumour size on chemotherapy, within the limitations already discussed; (3) that the biochemical dedifferentiation of the paraprotein, as evidenced by the presence of Bence-Jones protein, reflects the dedifferentiation of the underlying tumour.

Factors which help to form a prognosis at clinical presentation include the levels of haemoglobin, serum albumin and urea, and the presence of Bence-Jones or other proteins in the urine; prognostic factors during the subsequent monitoring of the patient include the rate and degree of response to chemotherapy.

The value of monitoring myelomatosis by serum and urine paraprotein measurements is clearly established and during treatment changes in Bence-Jones proteinuria and rate of rise of paraprotein levels must be watched for, and awareness of the possible development of other malignant diseases such as reticulosarcoma must be maintained, especially after five years of chemotherapy.

Only by the use of more sensitive biochemical monitoring of cytotoxic therapy will the best survival rates be achieved.

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