PAPERS AND ORIGINALS

Immunocytoma o' Mice an' Men*

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"The best-laid schemes o' mice an' men gang aft a-gley." —To a Mouse, Burns.

The mature plasma cell of Fagraeus represents the end product of one of the best-laid schemes o' mice an' men. This scheme, whereby individual plasma cells can synthesize their own individual antibodies to meet a vast range of environmental challenges, would truly have pleased such an individualist as Burns. As he said, even the best-laid schemes gang aft a-gley. I am going to consider what can happen when a plasma cell precursor gangs a-gley and goes on to form a clone of cells which may be recognized as a plasmacytoma. Since a plasmacytoma has a specific histopathological identity, it seems a good idea to use the term of Heremans, immunocytoma, which can cover all the various patterns that can be taken by tumours capable of producing immunoglobulins.

In considering these, three fundamental concepts have evolved over the last decade: (1) the monoclonal concept, (2) the paraprotein level usually reflects the amount of immunocytoma, and (3) biochemical dedifferentiation parallels malignant dedifferentiation.

The combined teamwork of the Medical Research Council's Myeloma Trials (under the chairmanship initially of Professor Witts and now of Professor Dacie) has played an important part in establishing these concepts. The work I am going to describe would have been impossible without the teamwork of 14 centres (see Hobbs, 1969a) throughout Britain, and I would especially like to thank Dr. David Galton and Professor Ian Wootton for their support throughout, and all those others who have done so much hard work over the past six years.

1. The Monoclonal Concept

By Burnet's theory one plasma cell produces one antibody—that is, a single immunoglobulin. Marchalonis and Nossal (1968) have indeed shown that this is generally the case.

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Following on Porter's work, it is known that immunoglobulins have a basic structure composed of two heavy chains joined to each other at a link region, and then joined to two light chains, nearly always by sulphhydryl bonds (the one known exception is a subclass of IgA—Grey, Abel, Yount, and Kunkel, 1968). Six different classes of heavy chain are now known (γ, μ, α, ϑ, ϵ, and ϕ; see Fig. 1) and subclasses occur within these. Two major classes of light chain are known (κ or γ) and each of these shows several subclasses. A given immunoglobulin molecule will contain only light chains of a

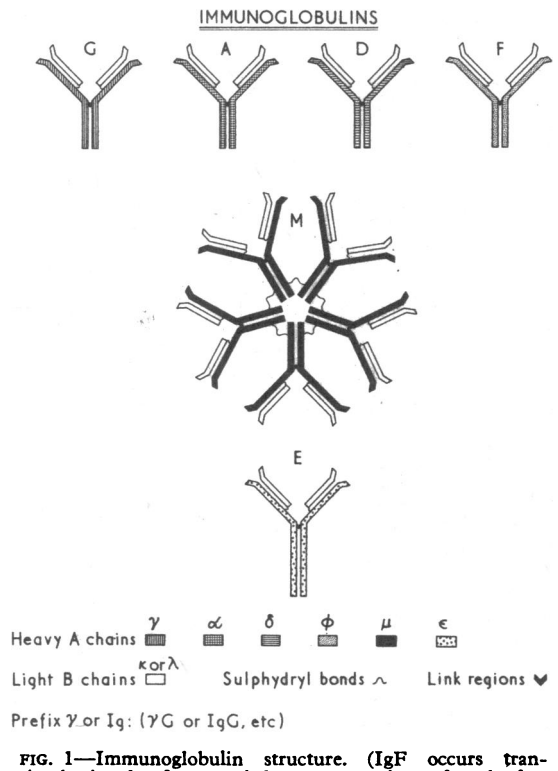

FIG. 1—Immunoglobulin structure. (IgF occurs transiently in the fetus and has not yet been found after birth.)

single class (κ or λ), whereas the normal mixture contains about twice as many κ as λ molecules.

Thanks to Waldenström (1962), we believe that if a single plasma cell precursor continues dividing to form a clone of cells all the daughter cells will eventually try to produce the same single immunoglobulin. If all the molecules have exactly the same structure they will share an identical electrophoretic mobility and will run as a single narrow band (see Fig. 2). On testing, this band will contain immunoglobulin determinants of a single subclass of heavy chain and/or a single subclass of light chain—that is, can be proved to be monoclonal. This is not the way normal antibody responses usually appear. In contrast, even a single hapten will usually be antigenic to the more than one clone of plasma cells in a given animal and will therefore elicit several antibodies. A single protein will usually contain several haptens and will elicit a spectrum of antibodies. A natural challenge—for example, diphtheria bacilli—more often presents many proteins, so that a broad spectrum of antibodies is elicited, usually containing most of the classes of heavy chains (γ, μ, α, ϑ, and ϵ) and light chains (κ and λ). On electrophoresis this spectrum will show a diffuse range of electrophoretic mobilities (from α_2 to γ_1, see Fig. 2) and can be recognized as polyclonal by its mixed content of immunoglobulin determinants.

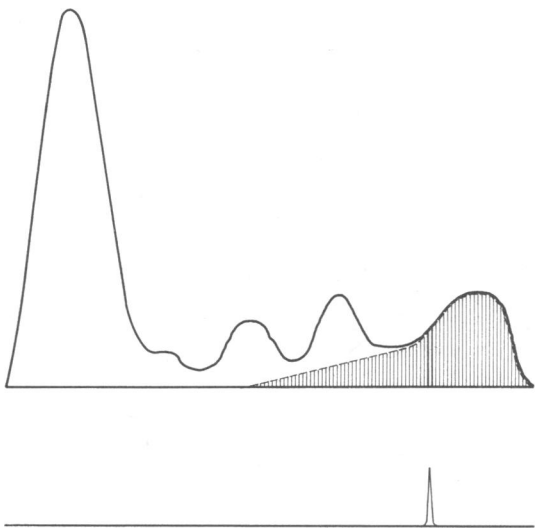

FIG. 2—Monoclonal concept. A paraprotein has a narrow electrophoretic mobility and contains heavy and/or light chains of a single subclass only. Polyclonal increases are broad and heterogeneous. (Reproduced, by permission of Athlone Press, from the *Scientific Basis of Medicine. Annual Reviews*, 1966.)

Thus when we find narrow bands on electrophoresis, and can identify them as being due to a single type of immunoglobulin, we call them paraproteins. We believe a paraprotein is evidence that a *monoclone* of cells is growing in the subject —that is, the subject has an immunocytoma. This cannot really ever be considered the normal scheme of things, something has gone awry. In medicine our concern then becomes whether or not such an immunocytoma is going to be harmful to its host, and thanks to studies in mice and men, we now monitor the growth of such clones by measuring paraprotein levels and look for evidence of dedifferentiation.

2. Paraprotein Level usually Reflects the Amount of Immunocytoma

With Potter's development of experimental plasmacytoma in mice it was established that the turnover of paraprotein (Nathans, Fahey, and Potter, 1958) or more simply the serum level (Osserman, Rifkind, Takatsuki, and Lawlor, 1964) was directly related to the weight of solid soft-tissue plasmacytoma. In our laboratory an ascitic form of plasmacytoma has been studied and by using isotope dilution it has been possible to estimate the actual total number of plasmacytoma cells in a mouse. At the same time the serum level of paraprotein was measured and a simple correlation was shown (Fakhri and Hobbs, 1970a) (see Fig 3). To the best of my knowledge this was the first time that the serum level of a tumour product had been directly related to the actually counted number of tumour cells. Incidentally, it was noted that the paraprotein could be first detected in the serum when a 23-g mouse had 3 million tumour cells. It was further shown that tumour growth was exponential in the mouse.

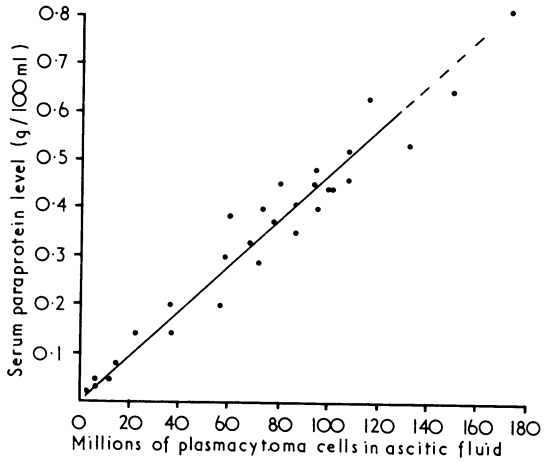

FIG. 3—Serum paraprotein level is directly proportional to the number of plasmacytoma cells, actually counted in mice with the ascitic tumour X5563. (Reproduced, by permission, from the *British Journal of Cancer*, 1970.)

While screening some 1,000 human patients, chiefly for the M.R.C. Myeloma Trials, we had the opportunity to follow 94 in whom a diagnosis was not initially certain, but who developed clear evidence of myelomatosis up to eight years later. We found that the rise of serum IgG or IgA paraprotein levels, or indeed the 24-hour urinary output of Bence Jones protein, was exponential, just as in the mouse. The median doubling time for eventual IgA myelomatosis our (most reliable estimate; Hobbs, 1969a) was 6.3 months. This is much slower than for normal antibody responses which can double in one day. Among all our cases of proved IgA myelomatosis the average serum level at clinical presentation was 2.8 g/100 ml. To maintain such a level in an average 70-kg patient would require the daily production of about 15 g of IgA paraprotein. Mammalian plasma cells on average produce 14 mg immunoglobulin/g cells/day, so that our average IgA patient should have about 1 kg of immunocytoma at clinical presentation. Now this figure is based on estimates of paraprotein production, etc.

With the help of Professor Hayhoe we derived another means of estimating the tumour mass. From all the marrow biopsies in all our patients he produced an estimate that 33% of the bone marrow cells were myeloma cells on average clinical presentation. A 70-kg patient could be expected to have 3·2 kg of bone marrow (Mechanik, 1926), and so we had an independent measurement confirming the previous estimate of about 1 kg of immunocytoma. This would be equivalent to $4{\cdot}6 \times 10^{11}$ tumour cells (see Fig. 4). With a little more than one further doubling this would become 10^{12} cells (identical to the estimate of the number of cells in acute leukaemia—Frei and Freireich, 1965) and death would follow.

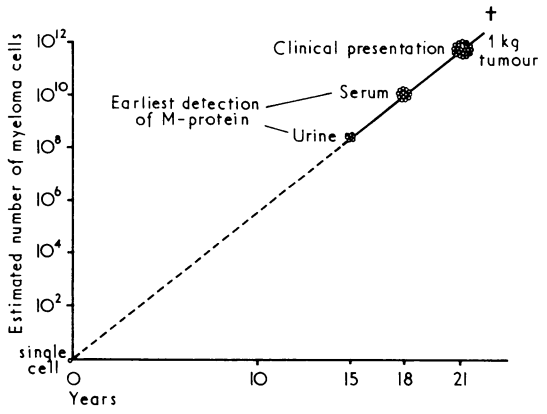

FIG. 4—Natural history of IgA myelomatosis. The log of the estimated number of tumour cells against time showed exponential growth for the solid part of the line. Serum IgA paraproteins are detected later than IgG, and so on average would be found only 2·6 years before clinical presentation $4{\cdot}6 \times 10^{11}$ tumour cells. Bence Jones proteinuria can be detected before this. The broken line is extrapolation back to a single cell, assuming a monoclonal origin and a constant growth rate.

This is in accord with observations that less than half the patients survived one year from presentation (Feinleib and MacMahon, 1960; Innes and Newall, 1961—before melphalan).

From the median doubling times, etc., it can be calculated that at the first chance that an IgG paraprotein can be detected in human serum there could be only some 20 g of immunocytoma (difficult to find, unless it is all in one vertebra, etc.) or some 9,000 million cells in our 70-kg patient. It is interesting to note that on a weight ratio (the mouse is 1/3,000 of man) this is like that actually found in the mouse. From such chance detection it would on average be five years before clinical evidence of myelomatosis emerged, and we have now indeed encountered eight such actually observed patients. By concentrating the urine some 300 times it is possible confidently to detect Bence Jones protein at a level of 1 mg/100 ml of original urine, or some 14 mg/day. This could represent as little as 3 g of immunocytoma, and this we may have done in three patients. Two of these have had their malignant immunocytoma verified by biopsy and clinical progress six and eight years later, and the third has developed three discrete osteolytic lesions in her clavicles at five years from the initial observation. The solid portion of the line in Fig. 4 is therefore observed fact. The broken line is a back extrapolation, assuming a constant doubling time (overall growth rate) as observed in the mouse.

From our studies of plasmacytoma in mice and men it seems that it takes some 21 years from the time the single plasma cell precursor goes awry for clinical IgA myelomatosis to become apparent. Our brief clinical view of the disease before death is only the tip of this chronological iceberg, which has important implications in treatment. My original estimate for IgG myelomatosis was 39 years (Hobbs, 1967), later modified to 33 years (Hobbs, 1969b) when more data became available. Normal polyclonal IgG shows an increased catabolic rate with rising serum level. Idiotypic IgG paraprotein might behave similarly. so that doubling times would appear longer and the above estimates could be too long, but then exponential increases would not be found and more curving should have been seen in log plots against time; furthermore, Drivsholm (1964) did not find changes in catabolic rates. It is comforting that we have encountered only one patient with IgG myelomatosis under the age of 33 years (he was 29, and his tumour had a fast growth rate). Salmon and Smith (1970) studied IgG metabolism in 10 patients and produced estimates for tumour cell masses of $0.5—3.1 \times 10^{12}$ in IgG myelomatosis. They stated that this is compatible with a natural history of 20 years. For myelomatosis producing Bence Jones protein only, faster growth has been estimated (Hobbs, 1969b), further confirmed by finding such patients under the age of 33 years (one under 20). Doubling times of one month have been observed and would allow Bence Jones mutations to emerge within three years of treatment (see below).

Now not all immunocytomata become clinical myelomatosis, some remain benign, and our studies can help in assessing the prognosis.

3. Biochemical Dedifferentiation Parallels Malignant Dedifferentiation

Firstly, we should consider synthesis of whole immunoglobulin. The heavy chain is synthesized on a large polyribosome and pulse labelling indicates this takes two and a half minutes. The light chain is synthesized on a smaller polyribosome, taking one minute (Askonas and Williamson, 1967). Assembly follows, the Golgi apparatus adds carbohydrate, and intact molecules are secreted only some 30 minutes later. Free light chains cannot be detected outside such plasma cells, and there is only a small intracellular pool of free light chains (presumably from the initial one and a half minutes before heavy chains are available). We therefore presume that light and heavy chain synthesis is beautifully balanced in the well-differentiated cell.

It had been thought that Bence Jones proteins were breakdown products of myeloma proteins. This was disproved when Putnam and Miyake (1958) injected glutamate into a patient with myelomatosis and found a much higher specific activity in the Bence Jones protein than in the myeloma protein. We now had a concept of de novo synthesis, and this I have taken further to a concept of biochemical dedifferentiation. The malignant myeloma cell has acquired an imbalance in heavy and light chain synthesis, presumably by dedifferentiation, and often produces too many light chains. These are secreted, and such free monoclonal light chains are indeed Bence Jones proteins. In some 20% of myelomatosis the process of dedifferentiation goes further and only light chains are synthesized and released. Such Bence Jones myelomata grow faster, can present at a younger age, seem more invasive in that more extensive bone and soft-tissue lesions are found, and carry a worse prognosis (Hobbs, 1969b); in short, they

TABLE I—*Immunoglobulin Fragments identified with Human Malignant Immunocytomata*

	Diseases	References
Light chains:		
(1) Bence Jones proteins	Various tumours	Hobbs (1969c)
(2) Half-light chains	Myelomatosis	Solomon *et al.* (1965). Wetter (1970)
Heavy chains:		
(3) γ	Lymphomata	Franklin (1970)
(4) α	Lymphomata	Seligmann *et al.* (1968)
(5) μ	Lymphomata	Tanigaki *et al.* (1966). Franklin (1970)
Incomplete molecules:		
(6) Half-molecules	Soft-tissue plasmacytoma	Hobbs and Jacobs (1969)
(7) 7s IgM	Various tumours	Carter and Hobbs (1971)
(8) Of IgG	Myelomatosis	Snigurowicz *et al.* (1968)

show that more biochemical dedifferentiation parallels more malignant dedifferentiation. If this is taken further, a few myelomata produce half-light chains and/or small amounts of Bence Jones proteins relative to the tumour mass—for example, down to one-fiftieth of the average 24-hour daily output of some 6 g at clinical presentation.

Fluorescent study of such bone marrow shows myeloma cells with a light chain content well below that of a normal plasma cell. Others even fail to produce any recognizable heavy or light chain at all; the so-called non-paraprotein myelomata. These latter groups clinically appear even more vicious than Bence Jones myelomata, and six out of eight such patients died within six months (four within three months) of diagnosis, despite our best treatment. Rarer tumours can fail to produce light chains, and only heavy chains are found. Still others produce only half-molecules found with soft-tissue plasmacytoma in both mice (Potter and Kuff, 1964) and men (Hobbs and Jacobs, 1969), for we have now found three such patients. IgM is normally secreted as a 19s molecule, assembled intracellularly from five 7s IgM units. After the initial reports of Solomon and Kunkel (1967), Bush, Swedlund, and Gleich (1969), and Hansson and Laurell (1969) a broader survey suggests that excess secretion of 7s IgM in the adult is probably evidence of malignant dedifferentiation (Carter and Hobbs, 1971). Further fragments of immunoglobulin synthesis and combinations of these continue to be reported, but almost throughout such dedifferentiated immunoglobulin synthesis has been found only with malignant immunocytomata (see Table I).

Our best evidence of this is a three-year follow-up of 402 patients in whom Bence Jones protein had been detected in our laboratory. Dr. Corbett obtained biopsy evidence of malignant immunocytoma in 400. The various forms such malignant immunocytomata can take are listed in Table II, which also includes the benign varieties in which I personally have not yet found immunoglobulin fragments.

TABLE II—*Diagnoses Achieved in 691 Patients with Paraproteins*

A.	**MALIGNANT IMMUNOCYTOMATA**		74%
	Myelomatosis (including 5 plasma cell leukaemias)	420	
	Waldenstrom's macroglobulinaemia	32	
	Soft-tissue plasmacytoma	20	
	Lymphosarcoma	26	
	Reticulosarcoma (including 5 atypical Hodgkin's)	6	
	Chronic lymphatic leukaemia (sclerotic myeloma)	5	
	Atypical myelosclerosis	2	
	Giant follicular lymphoma	1	
	Arabian lymphoma of gut (α-chain)	3	
B.	**BENIGN IMMUNOCYTOMA**		23%
	(1) Followed up for at least 5 years	112	
	(2) Monoclonal antibody:		
	Primary cold agglutinins (8 others had lymphoma)	37	
	Lichen myxoedematosis	4	
	Transient paraproteins	5	
C.	**UNCERTAIN**	18	3%

Together with this tendency for their immunoglobulin synthesis to often go awry, malignant immunocytomata also seem capable of suppressing the synthesis of normal immunoglobulins, so that with IgG myelomatosis the paraprotein band seen after electrophoresis on cellulose acetate usually sits on a white background because so little normal IgG remains. Levels of IgA and IgM are usually markedly subnormal. The serum level of paraprotein from malignant immunocytomata also shows a continued aggressive rise (so is usually above 1 g/100 ml) with time, whereas by the time we find paraprotein in benign immunocytomata the tumour seems to have already reached equilibrium with its neighbours and the serum level (often less than 1 g/100 ml) usually remains constant.

The four features that are very useful in predicting a benign or a malignant immunocytoma are shown in Table III, in order of importance, and wherever there is doubt we follow up the patient at yearly intervals (watching for feature 4). It is worth mentioning here that in over 1,000 patients with paraproteins, where such criteria have been followed, I am as yet aware of only one case I labelled as benign becoming malignant. That patient was given *x*-irradiation, and a paraprotein level which had been steady for five years suddenly rose rapidly three years after the treatment and Bence Jones proteinuria appeared for the first time. Evidence is given below that our current treatments can themselves be mutagenic and induce malignant dedifferentiation, and for this reason we like to be quite certain that an immunocytoma is malignant before using such measures. To diagnose myelomatosis, for example, we like to have a positive bone marrow biopsy (not just an excess of plasma cells, but cells which look neoplastic) and positive radiology (not just osteoporosis, common enough over 50 years of age, but localized areas of rarefaction) as well as positive protein studies (see Table III).

TABLE III—*Biochemical Features of Value in the Prognosis of Immunocytomata*

	Patients with Immunocytomata	
	517 Malignant (Biopsy Proved)	112 Benign (Followed for 5 years)
1. Immunoglobulin fragments	84%	0%
2. Suppression of normal immunoglobulins	98%	10%
3. Serum paraprotein level >1 g/100 ml	92%	15%
4. Progressive rise in paraprotein level	(Of those followed up untreated) 99%	1%

In some 90% of the patients in the M.R.C. Myeloma Trials all three criteria have been satisfied, and in the remainder at least two.

Treatment of Myelomatosis

Turning now to the treatment of the commonest malignant immunocytoma, myelomatosis, I do not have time to consider the important symptomatic treatments of hypercalcaemia, maintaining a high fluid throughput and keeping the patient

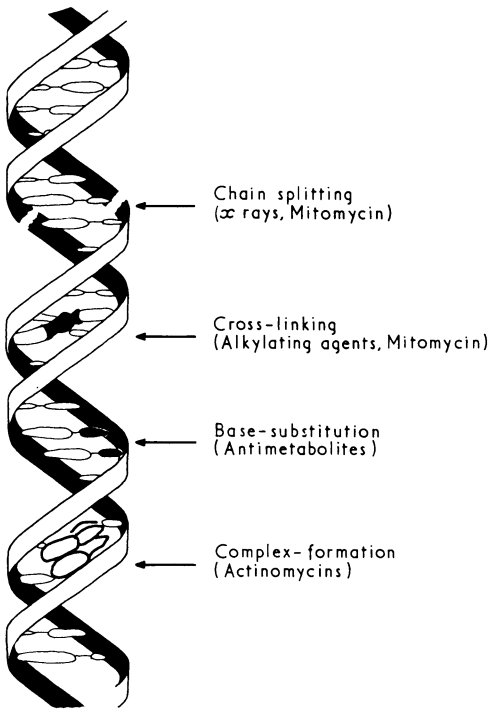

FIG. 5—To show how current antitumour treatments act on DNA. (By courtesy of Dr. M. Berenbaum.)

out of bed when possible. All our current cytotoxic treatments act through impairing DNA (Fig. 5) and it is not really surprising that the first M.R.C. trial showed no difference between melphalan and cyclophosphamide (Galton and Peto, 1968). These drugs mostly act on dividing cells, and because of the slow growth rate of myelomatosis it is probable that only some 5% of tumour cells are vulnerable at any one time. The treatment must therefore be prolonged, indeed it would seem for life.

The use of high dosage (1 mg/kg body weight) for four to seven days ensures that all dividing cells will be hit. This is then followed by a pause for six weeks, which enables the more rapidly dividing normal bone marrow cells, etc., to make a good recovery. Meanwhile the myeloma cells barely grow back at all. Another short period of high dosage is used, and the whole process continues to whittle down the myeloma cells, while allowing the normal cells to recover in the intermissions. This high-dosage intermittent treatment seems to be better than low-dosage daily treatment (Alexanian, Bergsagel, Migliore, Vaughn, and Howe, 1968), and the second M.R.C. trial is comparing the two, together with a third intermittent schedule, including prednisone.

The very fact that DNA is altered by the treatments allows the possiblity of inducing mutations, so that even another scheme of men could go awry. The first M.R.C. Myeloma Trial is now in its sixth year, and evidence of mutagenesis is collecting. Nevertheless, before presenting it, let me say at once that the skilled haematologically and biochemically controlled use of cyclophosphamide or melphalan (which owe much to the use of mouse plasmacytoma in their development) has been the single greatest advance to date in the treatment of human myelomatosis. I would go further and say that of all the general treatments of metastasized human cancer, it probably has produced the greatest reward in that some 50% of patients with this, the commonest, leukaemia are given two to three extra years of much less painful and useful life.

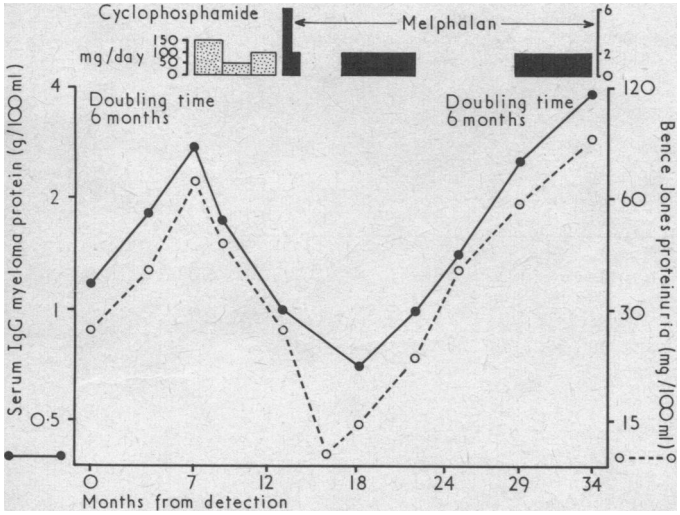

FIG. 6—*Simple escape* from the cyotoxic treatment of myelomatosis, at the same growth rate and with the same proportion of Bence Jones proteinuria.

How the application of concept 2 actually enables us to prove this is shown in Fig. 6. A patient observed before treatment was instituted had an IgG paraprotein with a doubling time of six months. Treatment reduced the level, which was not again reached for two years (at 31 months), when the tumour grew on to kill the patient three months later. The doubling time and the proportion of Bence Jones to serum paraproteins remained the same, and so this has been termed *simple escape* from treatment. This occurred in 55% of 87 patients who showed an initial response to cytotoxic treatment.

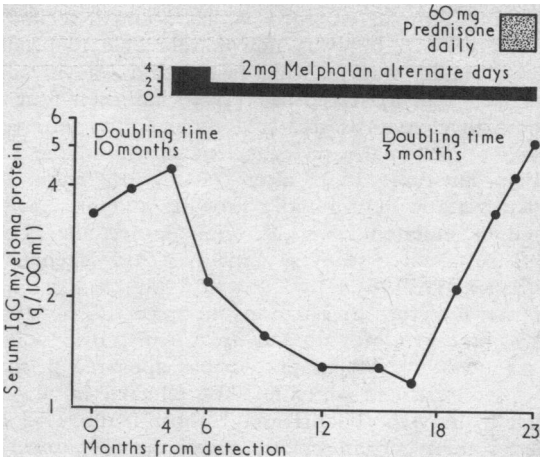

FIG. 7—*Growth rate escape*. During the relapse the serum paraprotein level doubles faster.

The other 45% all showed evidence of a change in their tumour, presumably induced by the treatment as no similar changes have yet been encountered as spontaneous events in over 100 initially untreated patients. All these changes (see Table IV) included an increase in growth rate of the tumour. This might be the only change (called *growth rate escape* in Fig. 7), but more often there was a disproportionate increase in the amount of Bence Jones proteinuria, or this even appeared for the first time (*Bence Jones escape* described elsewhere, Hobbs, 1969b). In some cases one of a mosaic of paraproteins was selected from patients showing more than one clone. In other patients a new but related paraprotein (from the same tribe) appeared (see Hobbs, 1969b), and in the case of Bence Jones proteins a second band has been shown to be one step removed (by a single amino-acid change —that is, a single mutation) from its parent clone (Wetter, 1970). This is also well described for mice (Laskov and Scharff, 1970).

TABLE IV—*Modes of Escape from Treatment in 87 Patients with Myelomatosis Who Initially Responded to Treatment, then Relapsed and Died*

Simple escape	55%	Mutation escape	5%
Growth rate escape	2%	Non-paraprotein escape	3%
Bence Jones escape	35%		

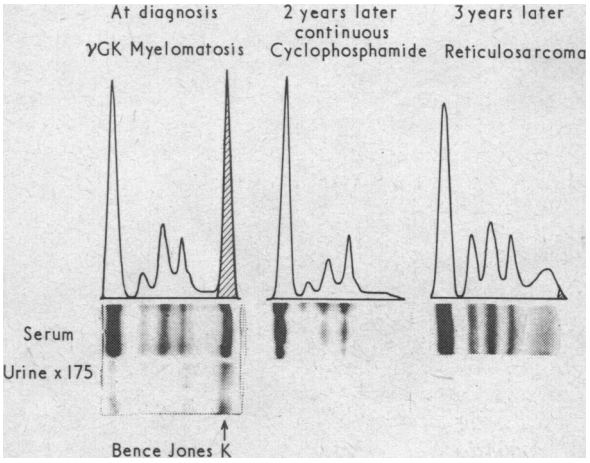

FIG. 8—*Non-paraprotein escape*. After complete disappearance of paraproteins from serum and urine, and recovery to a normal immunoglobulin pattern, a rapid relapse from typical myelomatosis was associated with a primitive tumour resembling a reticulosarcoma. A trace of the original paraprotein suggests the tumours are related. (By courtesy of Dr. J. Holt.)

In a few patients dedifferentiation was more complete, and a primitive tumour emerged which seemed related to the original myeloma, but was now barely able to produce any immunoglobulin, was growing faster, and was invading soft tissues (see Fig. 8). Osserman (1969) has seen four patients in whom myelomatosis treated for more than four years was followed by terminal monocytic leukaemia. All these changes largely occur three years after starting treatment, a period commensurate with a rapidly growing tumour having been selected or mutated from the original myeloma. Thus after some three years or so even man's best-laid scheme of treatment goes awry.

We are therefore investigating in mice the possibilities of adding immunotherapy to our treatments. Briefly it can be said that by the time paraprotein has appeared in the serum an immune treatment which is 100% effective in vitro is much less useful in vivo (Fakhri and Hobbs, 1970b) and certainly no better than current cytotoxic treatment. Applied in vivo at a time when the serum paraprotein level is just barely detectable it achieves a powerful reduction in the number of tumour cells (100,000 to 24) with a remission of some 10 days in our mice, equivalent to about 14 years in man. I hope we will be able to develop methods whereby in the lucky 15% of human patients in whom cytotoxic treatment renders the serum paraprotein invisible we may be able to add immune treatment and obtain a much longer remission.

Conclusion

I have tried to show how studies of plasmacytoma in mice and men have established three important concepts: (1) the monoclonal concept, (2) the paraprotein level usually reflects the amount of immunocytoma, and (3) biochemical dedifferentiation parallels malignant dedifferentiation. These have been of great value in our understanding, and in the diagnosis and treatment (especially in its monitoring) of myelomatosis and allied diseases. We have come a long way, but, having seen how the best-laid schemes of mice and men often go awry, you will realize we still have far to go.

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