Clin. exp. Immunol. (1976) 25, 428–436.

Intracytoplasmic and surface-bound immunoglobulins in 'nonsecretory' and Bence-Jones myeloma

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(*Received* 9 *February* 1976)

SUMMARY

Immunoglobulins were studied at the cellular level by direct immunofluorescence in twenty-five patients with 'nonsecretory' myeloma and thirty-six patients with Bence–Jones (BJ) myeloma. The results were compared with those obtained in a control group of thirty-six patients with common secretory myeloma. A monoclonal Ig (IgG in eighteen, IgA in three and κ chains only in three cases) was found in the cytoplasm of the plasma cells from all the patients with 'nonsecretory' myeloma, with a striking dysbalance in the staining brightness for the heavy and the light chains. A similar dysbalance in staining was also observed for plasma cell surface Ig chains but in the opposite way. In twenty patients with BJ myeloma studied for cytoplasmic Ig only, determinants of a heavy chain were clearly found in four cases. When surface Ig were studied also, the production of γ chains in addition to the light chain could be ascertained in six of sixteen cases. In addition, IgM with the same light chain type as the BJ protein was detected at the cell surface on plasma cells and lymphocytes in two of these sixteen patients. 'Monoclonal' populations of B lymphocytes bearing the same Ig chains as those produced by the myeloma cells were detectable in five of eleven 'nonsecretory' myeloma and in five of sixteen BJ myeloma patients. Normal blood B lymphocytes were in decreased number, particularly when a 'monoclonal' lymphocytic population was detected. Data are discussed which suggest that plasma cells from most patients with 'nonsecretory' myeloma might synthesize and secrete Ig molecules with structurally abnormal chains that are then quickly degraded.

INTRODUCTION

Careful studies of serum and urinary immunoglobulins (Ig) fail to show any monoclonal Ig in about 1% of patients with multiple myeloma (so-called 'nonsecretory' myeloma) whereas only monoclonal free light chains without complete monoclonal Ig are found in about 20% of myeloma cases (Bence–Jones (BJ) myelomas) (Osserman, 1965; Carbone, Kellerhouse & Gehan, 1967; Hobbs, 1969). The nature of the abnormality of Ig synthesis and secretion in 'nonsecretory' myeloma is yet unknown. In our initial study (Hurez, Preud'homme & Seligmann, 1970) and in several subsequent patients with 'nonsecretory' myeloma (Menkes *et al.*, 1972; Prost *et al.*, 1973; Arend & Adamson, 1974; Taylor & Mason, 1974; Whicher, Davies & Grayburn, 1975; Stites & Whitehouse, 1975), the presence of a monoclonal Ig within the proliferating plasma cells has been demonstrated by immunocytochemical methods. In contrast, immunofluorescence studies yielded negative results in some other cases (Gach, Simar & Salmon, 1971; River, Tewsbury & Fudenberg, 1972; Bedou *et al.*, 1974). It seemed therefore of interest to study by direct immunofluorescence cytoplasmic Ig in plasma cells from a large series of patients followed during the last 8 years. In addition, surface membrane Ig (SIg) of bone marrow cells and blood lymphocytes were examined in the most recently studied patients. On the other hand, the hypothesis that

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plasma cells from BJ myeloma patients could synthesize apparently unreleased heavy chains led us to perform similar experiments in this form of myeloma.

We wish to report here the results obtained in the study of twenty-five patients with 'nonsecretory' myeloma and thirty-six patients with BJ myeloma. As a control, the cells from thirty-six patients with myeloma cells secreting complete Ig molecules were also studied for intracytoplasmic and surface Ig.

MATERIALS AND METHODS

Patients. Only patients with an undoubted diagnosis of multiple myeloma (documented upon the basis of bone lesions and haematological findings) are included in this study. Repeated studies of serum proteins included search for cryoglobulins, electrophoresis, immunoelectrophoretic analysis using a variety of antisera polyvalent and monospecific for each Ig heavy and light chains and quantitative determinations of serum Ig levels by the radial immunodiffusion technique. Urinary proteins were concentrated 100–500 times by negative pressure dialysis and submitted to electrophoretic and immunoelectrophoretic analyses. 'Nonsecretory' myeloma was considered when the absence of any monoclonal Ig or BJ protein was consistently proven at repeated examinations of serum and concentrated urines. However, in five of the patients in the present series, trace quantities of BJ proteins were intermittently found in urines. These patients were included in the group of 'nonsecretory' myeloma since most other studies of their urinary proteins yielded negative results. The high number of patients with 'nonsecretory' myeloma in this study is not informative with respect to the incidence of this form of the disease. Indeed these patients were referred to us by a number of colleagues because of the lack of detectable monoclonal Ig and therefore correspond to a highly selected group.

Before classifying a case as BJ myeloma, careful attention was paid to rule out a rare complete Ig, particularly IgD. Common secretory myelomas in the control group included IgG myelomas (sixteen cases), IgA myelomas (fourteen cases), double IgG+IgA myelomas (two cases), IgM myelomas (two cases) and IgD myeloma (one case). Some results of the study of these patients have been reported elsewhere (Seligmann, Preud'homme & Brouet, 1973; Preud'homme & Seligmann, 1974; Preud'homme, Brouet & Seligmann, 1975).

In most patients, immunofluorescence studies were performed at the time of diagnosis and prior to therapy. We avoided studying patients receiving cytotoxic drugs for more than a few months, except for a few patients with 'nonsecretory' myeloma well documented before treatment and for some patients in whom changes in the serum and urinary Ig similar to those described by Hobbs (1971) were observed: patient 6 in Table 1 first had a classical BJ myeloma with a κ type BJ protein in serum and urines. This abnormality was no longer detectable after 1 yr of treatment and during the 2 following years, in spite of persistent bone marrow plasmacytosis and bone lesions. Trace amounts of κ type BJ protein were intermittently found at subsequent examinations of the patient's urines. In patient 14 in Table 1 a good remission was obtained by Melphalan therapy with a rapid disappearance of an IgGκ serum protein. A relapse occurred 16 months later without reappearance of the monoclonal IgG. The clinical course in these two patients has been previously reported (Menkes *et al.*, 1972; Prost *et al.*, 1973). Patient 11 in Table 3 had been receiving cytotoxic drugs for 7 years at time of study. At first examination and prior to therapy, her serum contained a monoclonal IgGλ that was barely detectable 1 year later and could not be detected any more at repeated subsequent examinations. An urinary BJ protein of the λ type was present throughout the course of the disease. When the immunofluorescence study was performed, an aggravation was apparent with increasing osteolytic lesions, bone marrow plasmacytosis and urinary BJ protein excretion.

Blood lymphocytes SIg were mostly studied in untreated patients because of the lack of reliability of figures obtained in patients who received cytotoxic or corticosteroid drugs.

Immunofluorescence studies. The methods used for the processing of bone marrow and blood cells and for their staining in direct immunofluorescence tests have been described in detail elsewhere, as well as the procedures used for the preparation of the anti-Ig reagents, their characteristics and the controls of their strict specificity at the level of sensitivity of membrane immunofluorescence (Preud'homme & Seligmann, 1972; Preud'homme & Labaume, 1975). Conjugates monospecific for γ, α, μ, κ and λ chains were used in all instances and a conjugated antiserum to δ chain in the most recently studied cases. Rhodamine-coupled antisera were used for the detection of SIg on living cells, whereas fluorescein conjugates were the usual reagents for staining cytoplasmic Ig in fixed cells. When studying bone marrow samples for SIg, plasma cells were identified on morphologic criteria in phase contrast microscopy and by double staining for cytoplasmic Ig using an antiserum coupled to the opposite fluorochrome.

RESULTS

'Nonsecretory' myeloma

In every patient, the presence of a monoclonal Ig within the cytoplasm of the proliferating plasma cells was easily demonstrated by cytoplasmic staining. This monoclonal Ig was IgGκ in sixteen cases, IgGλ in two, IgAκ in one, IgAλ in three and κ without detectable heavy chains in three cases. A surprising finding in this study was that of a strong staining for one Ig chain only (heavy or light) contrasting with a

TABLE 1. Nonsecretory myeloma. Immunofluorescence study limited to cytoplasmic staining

Patient number	Serum Ig (mg/ml)*			Cytoplasmic staining		Special features
	IgG	IgA	IgM	Strong	Faint or dubious	
1	14·0	1·8	0·54	α	λ	
2	5	0·34	0·1	α	λ	
3	4·8	0·4	0·3	κ	γ	
4	9·5	1·55	0·5	κ	γ	
5	17·8	2·2	1·9	κ	γ	
6	5·6	0·3	0·37	κ	0	Formerly BJ myeloma. Intermittent traces of BJ in urines
7	5·5	0·4	<0·1	κ	0	
8	5·2	0·57	<0·1	γ	κ	
9	7·4	1·2	0·5	κ	γ	Intermittent traces of BJ in urines
10	3·86	0·2	0·17	λ	γ	
11	2·6	1·17	0·2	λ	α	Amyloidosis. Intermittent traces of BJ in urines
12	2·8	0·15	<0·1	κ	0	
13	9·0	0·8	0·21	κ	γ	
14	5·6	0·29	0·13	κ	γ	Formerly IgG κ secretory myeloma

* Normal values: IgG, 11·7±2·7; IgA, 2·2±0·8; IgM, 1·2±0·5.

TABLE 2. Nonsecretory myeloma. Cytoplasmic and surface Ig

Patient number	Serum Ig (mg/ml)			Immunofluorescent staining											
				Bone marrow					Blood						
				Plasma cells				Lymphocyte surface	Lymphocyte surface						
	IgG	IgA	IgM	Cytoplasm		Surface			μ	δ	γ	α	κ	λ	
				Strong	Faint	Strong	Faint								
15	6·3	0·41	0·71	κ	γ	γ	κ	?	6		20	1	25	5	
16†	4·0	0·14	<0·1	κ	γ	γ	κ	?	<1	<1	<1	<1	<1	<1	
17	15·0	1·0	0·46	κ	γ	γ	κ	γκ	2	2	15	<1	16	1	
18†	22	2·13	7·8	κ	γ	γ	κ	0	<1	<1	<1	<1	<1	<1	
19		n.d.		κ	γ	κ	0	0	11		3	1	10	4	
20	9·8	1·2	0·42	κ	γ	γ	κ	γκ	3		23	<1	17	4	
21		n.d.		κ	γ	κ	γ	0	8		2	1	7	3	
22‡	18	2·3	1·4	κ	γ	All conjugates*		?	1		6	1	8	0	
23	5·9	0·2	<0·1	κ	γ	All conjugates*		?	<1		60	<1	60	<1	
24	7·8	0·33	0·46	γ	λ	γ	0	0							
25	1·6	0·17	0·17	α	κ	κ	0	?							

n.d.=Not determined. Very low levels of the three main Ig classes by immunoelectrophoresis.

Normal values for blood lymphocytes SIg: μ 5–25% (mean 11%), δ 5–12% (mean 8%), γ 1–7% (mean 4%), α 0·5–4% (mean 2%), κ 10–15% (mean 12·5%) and λ 1–7% (mean 4%).

* See text.

† Patients with intermittent presence of trace amounts of urinary BJ protein.

‡ Patient with bacterial endocarditis and polyclonal mixed cryoglobulin.

faint or dubious staining for the complementary light or heavy chain. This is in contrast with common secretory myeloma where we found a similar brightness for both chains in more than fifty patients. This disbalance in staining was striking in those patients whose plasma cells were studied for cytoplasmic Ig only (Table 1) and in several of these patients the dim staining for one chain was considered as meaningful only because it was consistently found when immunofluorescence studies were repeated several times.

The same observation was made in the patients whose cells were more recently studied both for cytoplasmic and surface Ig (Table 2). The findings for SIg provided a confirmation of the data obtained by cytoplasmic immunofluorescence. Indeed, the same Ig chains were found in the cytoplasm and on the plasma membrane of the plasma cells in most cases. Furthermore, the plasma cell SIg showed also a very unequal staining for the heavy and the light chains, but the pattern was usually the opposite from the one observed for intracytoplasmic Ig in the same patient, i.e. the chain that was barely detectable in the cytoplasm was strongly stained at the cell surface whereas conversely the chain strongly stained in the cytoplasm showed only a faint staining at the cell surface or was even undetectable, as in patient 25 in Table 2. In two patients, the plasma cell surface was labelled by all anti-Ig conjugates. This apparently nonspecific staining was observed in 17% of all cases of myeloma studied by us and is not yet clearly explained (Preud'homme *et al.*, 1975).

SIg on bone marrow lymphocytes could be appreciated in six cases and most lymphocytes bore the same Ig chains as the plasma cells in two of these patients. In the other cases (indicated by a question-mark in Table 2), the bone marrow samples did not contain enough lymphocytes to draw any conclusion. SIg on blood lymphocytes were studied in nine cases. A monoclonal population of lymphocytes that bore the same Ig chains as the plasma cell cytoplasmic Ig was found in five cases. This population accounted for a small percentage of blood lymphocytes in some patients in whom it was detectable only because of the very low figures for the other SIg chains. In other cases, a fair number of blood lymphocytes (as much as 60%) belonged to such a lymphocytic population (Table 2). The striking difference in the brightness intensity found for the heavy and the light chain in and on the plasma cells was not observed with the positive lymphocytes in the blood and in the bone marrow.

Bence-Jones myeloma

The main finding in the patients affected with BJ myeloma was the presence of apparently unreleased heavy chains in the bone marrow plasma cells from several patients. The immunofluorescence study was restricted to cytoplasmic Ig in twenty cases and in sixteen of these patients we could only detect monoclonal light chains. In the four other patients, cytoplasmic staining revealed the presence of heavy chain determinants of the γ (two cases) or α (two cases) class.

The incidence of cases with cellular heavy chains appears much higher among the sixteen further patients in whom SIg were also studied (Table 3). In two of these patients, γ chain determinants were easily found by cytoplasmic staining, with a brightness quite similar to that observed for the light chains. In four further patients, cytoplasmic staining showed a faint or dubious staining for γ chains which would have been easily missed if surface staining had not revealed a strong and obvious positivity for membrane-bound γ determinants. This observation was substantiated in two of these four cases by the finding of a population of bone marrow and blood lymphocytes that carried the same two Ig chains. In the two last patients in Table 3, only light chains were detected in the plasma cell cytoplasm whereas these plasma cells, most bone marrow lymphocytes (and most blood B lymphocytes in case 16) bore a monoclonal IgM with the same light chain type.

In this series of sixteen patients with BJ myeloma, 'monoclonal' lymphocytic populations with the same Ig chains as the plasma cells were found in the bone marrow and/or the peripheral blood in five cases. It may be of interest that such 'monoclonal' lymphocytes were found only in patients whose plasma cells produced either a monoclonal IgG or a monoclonal IgM and never in the more common cases with Ig synthesis apparently restricted to light chains. As in 'nonsecretory' myeloma, the brightness intensity of staining on the lymphocytes was quite similar for the heavy and the light chain.

'Monoclonal' lymphocytic populations were detected in the blood in 32% and in the bone marrow in

TABLE 3. Cytoplasmic and surface Ig in BJ myeloma

Patient number	Bone marrow: Plasma cell: Cytoplasmic Ig	Bone marrow: Plasma cell: SIg	Bone marrow: Lymphocyte: SIg	Blood lymphocyte SIg: μ	δ	γ	α	κ	λ
1*	γ++ λ++	0	0	<1	<1	<1	<1	<1	<1
2	γ++ κ++	κ	$\gamma\kappa$	6		1	2	5	3
3	κ++ γ+	γ++ κ+	$\gamma\kappa$	2	3	13	1	15	3
4	λ++ $\gamma\pm$	γ+++ λ++	$\gamma\lambda$	3	1	28	<1	2	30
5	λ++ $\gamma\pm$	γ++ λ+	0	2	6	3	1	8	3
6	λ++ $\gamma\pm$	γ++ $\lambda\pm$	0						
7	λ	0	0						
8	κ	0	0	3	2	<1	<1	2	1
9	κ	? all†† conjugates	?†	10		1		10	3
10	κ	κ	?†						
11*	λ	0	0						
12	λ	λ	0						
13*	κ	κ	?†	<1		<1	<1	<1	<1
14	λ	0	0	7		<1	<1	4	1
15	κ	$\mu\kappa$	$\mu\kappa$						
16	λ	$\mu\lambda$	$\mu\lambda$	9		1	<1	<1	9

* Patients 1 and 13: plasma cell leukaemia; patient 11: formerly IgGλ myeloma (see Materials and Methods section). Amyloidosis.

† See text.

30% of the thirty-six control patients with common secretory myeloma. The percentages of 'monoclonal' lymphocytes ranged from 4–46%. These figures do not differ from those observed in 'nonsecretory' and BJ myelomas. It should, however, be noted that monoclonal lymphocytes were found in both patients with IgM myeloma who were studied.

Reduced figures for normal SIg-bearing blood lymphocytes were observed in common secretory myeloma as in 'nonsecretory' and BJ myelomas, without any statistical difference between these three groups. Data available in the sixty-two patients with the various types of myeloma whose cells were studied for SIg could therefore be pooled and are indicated in Table 4. It is apparent that the patients with

TABLE 4. Mean percentages of normal SIg-bearing blood lymphocytes in multiple myeloma

	μ	δ	γ	α	κ	λ
All patients	4·0	2·0	1·4	0·5	4·3	2·3
Patients with a 'monoclonal' lymphocytic population	3·0	1·6	0·6	0·3	1·5	2·2
Patients without a detectable 'monoclonal' lymphocytic population	4·5	2·3	1·6	0·6	5·2	2·4
Normal controls (Preud'homme & Seligmann, 1972)	11	8	4	2	12·5	4

'monoclonal' lymphocytic populations had less circulating normal B lymphocytes than those without such populations.

DISCUSSION

Immunofluorescence study of bone marrow plasma cells from twenty-five patients with 'nonsecretory' myeloma showed in all cases a monoclonal Ig in the cytoplasm of the cells. This is in contrast with the absence of Ig detectable by cytoplasmic immunofluorescence in a few patients (Gach *et al.*, 1971; River, Tewsbury & Fudenberg, 1972; Bedou *et al.*, 1974). Although not confirmed by biosynthesis studies, these negative results suggest that the plasma cells from such patients may have lost the ability to synthesize Ig chains. *In vitro* studies of mutation in Ig production by mouse myeloma cells have shown that in certain cultured cell lines the cells convert from IgG producers to nonproducers at a very high rate of about 10^{-3}–10^{-4} per cell per generation (Baumal *et al.*, 1973; Cotton, Secher & Milstein, 1973). In other cell lines, a stepwise conversion is observed from IgG producers to light chain producers, then from light chain producers to nonproducers and the mutation rate of these events is also very high (Coffino & Scharff, 1971; Baumal *et al.*, 1973). If similar mutations occur *in vivo* in human patients, it is not known whether such mutant clones would overgrow the wild type cells because of some kind of selective advantage or would be quickly eliminated. In any case, the incidence of nonproducers in human myeloma is surely very low.

The finding of monoclonal Ig in the plasma cells is of course not informative with regard to the mechanisms responsible for the absence of monoclonal Ig in the serum and urines, and careful biosynthesis studies are required to get some insight into the pathogenesis of the so-called nonsecretion phenomenon. Since a number of variant clones originating from murine plasmacytomas have been studied, it is clear that the apparent nonsecretion may correspond at least to two distinct situations. Some variants are true nonsecretors and the lack of secretion is due either to intracellular accumulation of Ig chains followed by a decrease in their synthetic rate as in the variants producing apparently complete heavy chains but not light chains studied by Morrison & Scharff (1975) or to intracellular degradation of Ig molecules with abnormal (likely deleted) heavy chains (Cowan, Secher & Milstein, 1974). On the other hand, in mice bearing certain tumours producing Ig with abnormal heavy chains, there is a contrast between the absence of detectable serum monoclonal Ig and the findings of an active secretion by biosynthetic studies (Birshtein, Preud'homme & Scharff, 1974a,b). It is possible that in such cases secretion also occurs *in vivo* and is followed by a rapid degradation of the abnormal Ig molecules which could be more susceptible to enzymatic breakdown than normal Ig. Preliminary results of the study performed with J. Buxbaum of Ig synthesis and secretion after incorporation of radioactive precursors *in vitro* suggest that a similar situation occurred in several cases of human 'nonsecretory' myeloma. The finding of SIg on our patients' plasma cells argues in the same way. Indeed, in the mouse system, the true nonsecretors appear to be unable to incorporate SIg into their plasma membrane (Bailey, Hannestad & Eisten, 1973; Milstein *et al.*, 1974; Preud'homme & Morrison, unpublished data), a finding which is in accordance with the concept that the intracellular transport of SIg follows the same pathway as that of Ig molecules destined for secretion (Uhr & Vitetta, 1973). It is worth noting that in two cases the plasma cells first secreted a monoclonal IgG or free light chains and became apparent nonsecretors after Melphalan therapy, a drug known to be mutagenic for Ig production by murine plasma cells (Preud'homme, Buxbaum & Scharff, 1973). Some of the apparently nonsecretor murine mutant clones mentioned above were generated by mutagenesis with Melphalan.

The hypothesis that plasma cells from many patients with 'nonsecretory' myeloma secrete Ig moieties with an abnormal heavy or light chain which are then rapidly degraded would easily explain the absence of ultrastructural features suggestive of intracellular retention of proteins (Azar *et al.*, 1972), the finding of amyloidosis in one patient in this and another (Azar *et al.*, 1972) series and the detection in several cases of intermittent urinary output of minute quantities of BJ protein. The strikingly faint reactivity of one intracellular Ig chain with the conjugated antibodies found in our and other (Arend & Adamson, 1974) patients would be compatible with the hypothesis of a primary structure abnormality of this chain. The reason why the same Ig chain is strongly stained at the cell surface is unclear.

When the study of patients with BJ myeloma was limited to cytoplasmic immunofluorescence, the presence of heavy chain determinants could be ascertained only in a few cases. However, when SIg were also studied, the obvious positivity for the heavy chain on the plasma cell membrane—and eventually the finding of a lymphocytic population bearing the same Ig moieties—substantiated the observation of a weak positivity for this chain in the cytoplasm. In these conditions the plasma cells from about one-third of the patients with BJ myeloma seemed to produce apparently unreleased heavy chains. Heavy chains were also found in one of three cases of BJ myeloma studied by biosynthetic experiments (Zolla, Buxbaum & Tanapatchaiyapong, 1970) and in one of seven cases studied by the immunoperoxidase method (Taylor & Mason, 1974). The lack of detection of heavy chain containing molecules in the patients' serum or urines raises the same hypotheses as those discussed above for 'nonsecretory' myeloma. Intracellular degradation of the heavy chains has been documented in some variants of mouse myeloma cells which produce both heavy and light chains but secrete only light chains (Morrison *et al.*, 1974). An alternate possibility is that of a structural abnormality of the heavy chain leading to its proteolysis after it has been secreted. It is worth noting that the cells from the patient studied by Zolla *et al.* (1970) secreted IgG molecules *in vitro*.

The finding of surface IgM on the light chain containing plasma cells (and on 'monoclonal' lymphocytes) from two of our patients with BJ myeloma is not peculiar to this type of myeloma since IgM with the same light chains as the secreted monoclonal Ig has been found on IgG or IgA producing myeloma cells from a few patients (Seligmann *et al.*, 1973; Stein & Kaiserling, 1974). This finding is reminiscent of that of Pernis, Forni & Amanti (1971) for normal IgG plasma cells in the rabbit, an observation thought to be related to the switch from IgM to IgG or IgA synthesis at the level of a single clone. The BJ-secreting cells with surface IgM may be derived from such IgG or IgA plasma cells. Alternatively they may arise from IgM-producing plasma cells by a loss of heavy chain synthesis. The finding of IgM bearing 'monoclonal' lymphocytes in the two patients might favour this latter hypothesis.

In the present study, populations of lymphocytes which carried SIg with the same heavy and light chains as the plasma cells were found in five of eleven 'nonsecretory'—and five of sixteen BJ myeloma cases. This incidence does not significantly differ from that found in common secretory myelomas (Seligmann *et al.*, 1973, Preud'homme *et al.*, 1975). The actual synthesis of these lymphocyte 'monoclonal' SIg has been proven in some cases (Mellstedt, Hammarstrom & Holm, 1974) and these lymphocytes appear to be B cells upon the basis of these findings and of other B cell markers (C receptors, binding of IgG aggregates). They are thought to belong to the proliferating clone since they share the idiotype of the monoclonal Ig secreted by the plasma cells (Mellstedt *et al.*, 1974). These monoclonal lymphocytes are likely different from the more numerous lymphocytes that react with antisera to the monoclonal Ig idiotype but not with anti-Ig isotype sera (Lindstrom *et al.*, 1973; Chen *et al.*, 1975) and which may well not belong to the B cell series. A RNA-containing factor secreted by the plasma cells appears to be able to convert normal lymphocytes to express the idiotypic specificity of the monoclonal Ig (Giacomoni *et al.*, 1974; Chen *et al.*, 1975).

It is clear from Tables 2 and 3 that normal B lymphocytes were in reduced numbers in the patients' blood, confirming previous data in common secretory myeloma (Seligmann *et al.*, 1973; Lindstrom *et al.*, 1973; Mellstedt *et al.*, 1974; Knapp *et al.*, 1974; Chen *et al.*, 1975). In all varieties of myeloma, the percentages of normal B lymphocytes were clearly lower in the patients in whom a monoclonal lymphocytic population was detectable. We found no strict correlation between figures for blood B cells and serum Ig levels. Contrasting with the polyclonal hypoimmunoglobulinaemia almost constantly observed in common myeloma, normal or increased levels of normal serum Ig of all three main Ig classes were a striking feature in five of twenty-five patients with 'nonsecretory' myeloma (Tables 1 and 2). Careful electrophoretic and immunoelectrophoretic analysis of the patients' sera at various dilutions and of the serum IgM purified by density gradient ultracentrifugation failed to show evidence for any homogeneous component within the polyclonal Ig. Of course such studies cannot completely rule out the presence of a monoclonal fraction in extremely small amounts. Biosynthetic studies performed with bone marrow cells from one of these patients with diffuse hyperimmunoglobulinaemia showed the predominant production of the monoclonal Ig but also the synthesis of polyclonal Ig, a finding which

most uncommon in myelomas. In one of these cases, the serum contained a cryoglobulin which was composed of polyclonal IgG and polyclonal IgM. Some of these patients were affected with associated diseases (bacterial endocarditis, multiparasitic infestations...) where polyclonal hyperimmunoglobulinaemia is a common finding. The observation of increased levels of normal serum Ig in some patients with 'nonsecretory' myeloma should be considered when discussing the mechanism of the humoral immunodeficiency usually associated with neoplastic plasma cell proliferations, a complex mechanism which associates several factors (Zolla *et al.*, 1974; Giacomoni *et al.*, 1974; Broder *et al.*, 1975).

We wish to thank Mrs S. Labaume for her expert technical assistance. This work was supported by INSERM (contract no. 76.5.116.1).

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