

Association of plasma cell subsets in the bone marrow and free light chain concentrations in the serum of monoclonal gammopathy patients

Michael John Ayliffe,¹ Judith Behrens,² Simon Stern,² Nazira Sumar¹

¹Department of Immunology, Epsom and St Helier University Hospitals NHS Trust, Carshalton, Surrey, UK
²Department of Haematology, Epsom and St Helier University Hospitals NHS Trust, Carshalton, Surrey, UK

Correspondence to

Michael John Ayliffe, Department of Immunology, St Helier Hospital, Wrythe Lane, Carshalton, Surrey SM5 1AA, UK; michael.ayliffe@talktalk.net

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ABSTRACT

This study investigated bone marrow plasma cell subsets and monoclonal free light chain concentrations in blood of monoclonal gammopathy patients. 54 bone marrow samples were stained by double immunofluorescence to enumerate cellular subsets making either intact monoclonal immunoglobulin or free light chains only. Blood taken at the same time was assayed for free light chains by an automated immunoassay. Patients were assigned to three cellular population categories: single intact monoclonal immunoglobulin (59%), dual monoclonal immunoglobulin and free light chain only (31%), or single free light chain only (9%). The median affected free light chain concentration of each group was 75 mg/l, 903 mg/l and 3320 mg/l, respectively, but with substantial overlap. In myeloma patients the difference in serum free light chain concentrations between patients with free light chain only marrow cells and those without was statistically significant. Serum free light chain levels >600 mg/l result mostly from marrow cells restricted to free light chain production.

INTRODUCTION

Monoclonal gammopathies result from monoclonal proliferation of immunoglobulin (Ig) producing cells of B-cell lineage. They are characterised in the serum and/or urine by the presence of monoclonal immunoglobulin (M-Ig) of restricted heavy and light chain isotype and electrophoretic mobility. They are found in a variety of malignant conditions such as multiple myeloma, solitary plasmacytoma, amyloid light-chain (AL) amyloidosis and lymphoplasmacytic lymphoma. They also occur in individuals with no current manifestations of disease where they may represent a premalignant condition, monoclonal gammopathy of undetermined significance (MGUS). Free light chains (FLCs) of M-Ig can also occur in serum and/or urine where they can be quantified by automated immunoassays.¹

We have previously demonstrated dual populations of bone marrow plasma cells making intact M-Ig or restricted to monoclonal FLC production alone (FLC-only) in a proportion of individuals with multiple myeloma.² Changes were documented in subset populations within individual patients during disease and treatment. A separate subset of FLC-only cells was associated with shorter patient survival.

Sharp rises in the concentration of FLCs in serum (sFLCs) have been shown by others to be associated with disease progression, light chain escape and relapse in multiple myeloma.^{3–4} Published guide-

lines recommend measurement of sFLCs in risk stratification for MGUS, to measure response to therapy in light chain myeloma, and in monitoring AL amyloidosis and non- and oligo-secretory myeloma.^{5–7} We report the association between bone marrow plasma cell subsets and concentrations of sFLCs of individuals being investigated for monoclonal gammopathy.

DESIGN AND METHODS

The London–Surrey Borders Research Ethics Committee approved the study. All participants gave informed consent. Bone marrow samples were obtained from 54 patients presenting for investigation of monoclonal gammopathy. Plasma cell subsets in bone marrow were counted using a well established, reliable double immunofluorescence method as previously described.^{2–8} Briefly, cyto-centrifuge preparations of washed, fixed bone marrow cells were stained with different Ig heavy or light chain specific antisera conjugated to fluorescein isothiocyanate, to each of which were added a polyspecific anti-Ig conjugated to rhodamine. Plasma cells were identified by morphology and bright red cytoplasmic fluorescence, and the specific isotype of each plasma cell by apple green fluorescence using an appropriate different filter set. Seven hundred plasma cells were counted for each sample, 100 in each of the seven preparations. Plasma cells of each heavy and light chain type were thus counted independently. The procedure resembled immunofixation but applied at the cellular level. Samples containing FLC-only cells were identified by a discrepancy in the sum of the heavy and light chain isotypes. For example, for patient 37, a sum of IgG+IgM+IgA+IgD heavy chain percentages of 59% together with a sum of light chain (κ + λ) percentages of 100% indicated that 41% of cells were making λ FLCs only.

Blood collected within 6 h of the bone marrow aspiration was assayed for sFLCs on a Siemens BNII nephelometer by standard latex-enhanced immunoassays (Freelite; Binding Site, Birmingham, UK) following the manufacturer's recommended protocols.

Results were displayed by cellular population against involved sFLC concentration. Monoclonal gammopathies of IgM type differ clinically and morphologically from those of other isotypes, so serum and bone marrow findings were compared separately for multiple myeloma and lymphoplasmacytic lymphoma using the Mann–Whitney rank sum test.

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Table 1 Monoclonal plasma cell populations in the bone marrow and free light chain (FLC) concentrations in the serum of 54 patients with monoclonal gammopathy

Patient	Cell populations*	BM infiltration†	FLC only‡	sFLK (mg/l)§	sFLL (mg/l)¶	sFLKL ratio**	Paraprotein	Diagnosis
Single monoclonal cell population M-Ig								
1	M-Ig	12%	2%	613	3	226.20	IgGK 28 g/l	Asymptomatic MM
2	M-Ig	11%	<1%	104	13	8.19	IgGK 16 g/l	Treated MM
3	M-Ig	64%	<1%	41	9	4.69	IgGK 39 g/l	New MM
4	M-Ig	<10%	4%	41	20	2.08	IgGK 19 g/l	New MGUS
5	M-Ig	90%	2%	23	2	14.29	IgGK 29 g/l	New MM
6	M-Ig	40%	2%	612	7	90.27	IgGK 33 g/l	New MM
7	M-Ig	5%	<1%	688	68	10.16	IgGK 7 g/l	New MGUS
8	M-Ig	42%	<1%	1	1	1.00	IgGK 23 g/l	New MM
9	M-Ig	10%	<1%	14	2	6.10	IgGK 21 g/l	New MM
10	M-Ig	15%	<1%	23	106	0.21	IgGL 18 g/l	MGUS
11	M-Ig	83%	<1%	1	1090	<0.01	IgGL 45 g/l	New MM
12	M-Ig	15%	<1%	7	83	0.09	IgGL 17 g/l	Asymptomatic MM
13	M-Ig	17%	2%	42	208	0.20	IgGL 18 g/l	AL amyloidosis
14	M-Ig	10%	<1%	3	27	0.10	IgGL 14 g/l	Relapsed MM
15	M-Ig	18%	<1%	10	12	0.82	IgGL 14 g/l	MGUS
16	M-Ig	26%	<1%	2	332	0.01	IgGL 28 g/l	New MM
17	M-Ig	49%	2%	29	3	11.60	IgAK 23 g/l	New MM
18	M-Ig	31%	<1%	1480	1	1057.14	IgAK 13 g/l	Multiply relapsed MM
19	M-Ig	19%	<1%	33	<1	370.00	IgAK 12 g/l	Treated MM
20	M-Ig	33%	<1%	122	2	70.93	IgAK 51 g/l	New MM
21	M-Ig	28%	<1%	51	6	7.92	IgAK 17 g/l	New MM
22	M-Ig	80%	<1%	9	773	0.10	IgAL 84 g/l	New MM
23	M-Ig	20%	<1%	5	387	0.01	IgAL 16 g/l	New MM
Dual monoclonal cell populations; M-Ig and FLC only								
24	Dual M-Ig and FLC only	70%	13%	254	6	42.19	IgGK 28 g/l	New MM
25	Dual M-Ig and FLC only	50%	75%	15 300	16	944.45	IgGK 12 g/l	New MM
26	Dual M-Ig and FLC only	47%	10%	328	1	381.95	IgGK 50 g/l	New MM
27	Dual M-Ig and FLC only	70%	17%	1360	7	182.06	IgGK 17 g/l	New MM
28	Dual M-Ig and FLC only	40%	7%	1100	8	138.19	IgGK 50 g/l	New MM
29	Dual M-Ig and FLC only	10%	20%	14	223	0.06	IgGL 11 g/l	MGUS
30	Dual M-Ig and FLC only	19%	37%	23	801	0.08	IgGL 30 g/l	New MM
31	Dual M-Ig and FLC only	40%	91%	903	9	105.61	IgAK 2 g/l	New MM
32	Dual M-Ig and FLC only	20%	6%	0	275	0.01	IgAL 6 g/l	Treated MM
33	Dual M-Ig and FLC only	5%	7%	14	1970	0.01	IgAL 5 g/l	SP sternum
34	Dual M-Ig and FLC only	22%	23%	13	2640	0.01	IgDL 2 g/l	New MM
35	Dual M-Ig and FLC only	85%	53%	29 800	16	1862.5	Free KLCs	Light chain MM
36	Dual M-Ig and FLC only	70%	91%	34	17 100	0.01	Free LLCs	Light chain MM
37	Dual M-Ig and FLC only	60%	41%	13	15 500	0.01	Free LLCs	New MM, ARF
38	Dual M-Ig and FLC only	10%	58%	38	209	0.18	Free LLCs	FLC MGUS
Single monoclonal cell population; FLC only								
39	FLC only	55%	95%	4140	1	5376.62	Free KLCs	New light chain MM
40	FLC only	50%	92%	3320	7	481.86	Free KLCs	New light chain MM
41	FLC only	10%	92%	585	14	42.09	Free KLCs	AL amyloidosis
42	FLC only	20%	56%	61	629	0.1	Free LLCs	AL amyloidosis
43	FLC only	18%	66%	32	3340	0.11	Free LLCs	New light chain MM
IgM monoclonal gammopathies								
44	M-Ig	4%	<1%	67	6	11.18	IgMK 21 g/l	Untreated LL
45	M-Ig	20%	<1%	33	11	2.97	IgMK 36 g/l	New LL
46	M-Ig	Non-diagnostic	<1%	19	11	1.62	IgMK 20 g/l	IgM MGUS
47	M-Ig	16%	<1%	107	21	5.10	IgMK 11 g/l	New LL
48	M-Ig	42%	<1%	436	6	27.00	IgMK 12 g/l	Relapsed LL
49	M-Ig	<10%	<1%	22	10	2.18	IgMK 5 g/l	CHAD
50	M-Ig	29%	<1%	7	7	1.04	IgMK 20 g/l	New LL
51	M-Ig	20%	<1%	8	38	0.20	IgML 5 g/l	New LL
52	M-Ig	19%	<1%	12	101	0.10	IgML 16 g/l	New LL
53	Dual M-Ig and FLC only	62%	14%	36	13	2.02	IgMK 19 g/l	New LL
54	Dual M-Ig and FLC only	4% lymphocytes	45%	30	6	4.83	IgMK 5 g/l	Treated LGL, CHAD

*Monoclonal plasma cell populations found in marrow.

†Proportion of plasma cells in bone marrow.

‡Proportion of plasma cells in marrow making free light chains only.

§Concentration of free κ light chains in serum.¶Concentration of free λ light chains in serum.**Ratio of free κ to free λ light chains in serum.

AL, amyloid light-chain; ARF, acute renal failure; CHAD, cold haemagglutinin disease; LGL, low grade lymphoma; LL, lymphoplasmacytic lymphoma; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; SP, solitary plasmacytoma.

RESULTS AND DISCUSSION

There were 31 male and 23 female patients (ratio 1.3:1) with a median age of 73 years (range 40–88). The distribution of M-Igs by isotype was in agreement with published figures; IgG, 23/54 (43%); IgM, 11/54 (20%); IgA, 10/54 (19%); IgD, 1/54 (2%); κ light chain only, 4/54 (7%); λ light chain only, 5/54 (9%).⁹ The distribution by diagnosis was varied: multiple myeloma=33; AL amyloidosis=3; solitary plasmacytoma=1; MGUS=7; lymphoplasmacytic lymphoma=8; low grade lymphoma=1; cold haemagglutinin disease=2. Cell population bone marrow results together with paraprotein and sFLC assays are presented in table 1.

In 32/54 samples (59%), a single population of plasma cells staining for intact M-Ig were detected (M-Ig group). Although this cell population is characterised by M-Ig staining, 24/32 of them (75%) were also making FLCs as indicated by abnormal sFLC ratios in the appropriate direction. These intracellular FLCs were not separately demonstrable with this staining technique. A single population of FLC-only cells were demonstrated in 5/54 samples (9%): 3 multiple myelomas and 2 AL amyloidosis. In 17/54 samples (31%), dual populations of M-Ig and FLC-only plasma cells were seen together. Dual populations were always of the same light chain isotype and all had abnormal sFLC ratios in the appropriate direction. By immunofixation, M-Ig was found in the serum of 13 patients with dual bone marrow populations, but the remaining four had only free light chains, indicating a sensitivity difference between intracellular detection by immunofluorescence over electrophoretic detection of the secreted product in serum.

The distribution of the involved sFLC concentrations for each of the three cellular categories is displayed on a logarithmic scale to accommodate the wide range of concentrations (figure 1). For the three cellular categories, the median involved sFLC concentrations (K or L) were 75 mg/l for the M-Ig group, 903 mg/l for dual M-Ig and FLC-only, and 3320 mg/l for FLC-only, but with some overlap.

FLC-only cells were seen in the marrow of 11/34 multiple myeloma patients (32%) and in 2/10 of those with lymphoma (20%). One MGUS patient showed a dual population as did the solitary plasmacytoma. Two of the amyloidosis patients showed FLC-only cells alone. These findings confirm that FLC-only plasma cells are not rare in monoclonal gammopathies.

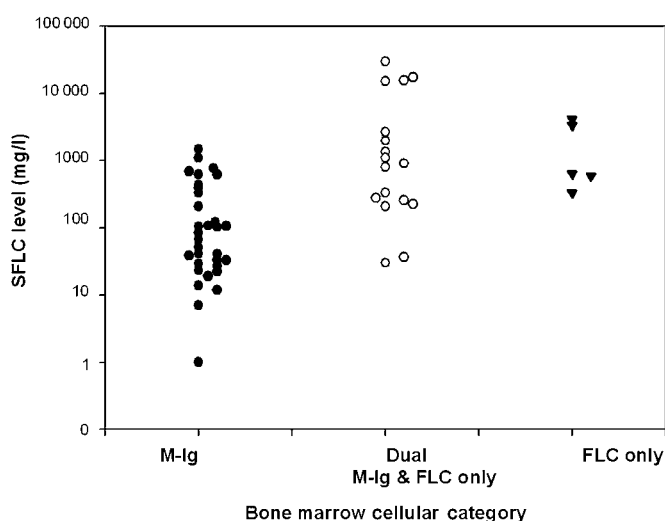


Figure 1 Relationship between the cell population category and serum free light chain (SFLC) concentration of involved light chains in monoclonal gammopathy patients.

Take-home messages

- ▶ A separate population of bone marrow plasma cells restricted to monoclonal free light chain production occurs in about a third of myeloma patients irrespective of the isotype of the intact M-Ig.
- ▶ Such cells also occur in a minority of other monoclonal gammopathies.
- ▶ The presence in the marrow of a population of FLC-only cells is significantly associated with increased concentrations of free light chains in the serum.

Across all three cellular categories there was no significant difference in the sFLC concentrations between patients with κ - and λ -restricted tumours nor between the dual and FLC-only cellular categories. For the 34 multiple myeloma patients (amyloidosis, solitary plasmacytoma and low grade lymphoma excluded) there was a significant difference in sFLC concentrations between the M-Ig and both dual and FLC-only patients ($p \leq 0.001$). This is consistent with the sFLC increases mainly being consequent on the development of an FLC-only marrow plasma cell population, and also with the bone marrow and serum observations being different aspects of the same process. In the few lymphoplasmacytic lymphoma patients no significant difference was detected.

These results show that bone marrow FLC-only cells are substantial contributors to raised levels of sFLCs, whether there are M-Ig cells present or not, and provide a cellular basis for most of the high levels of sFLCs. However, FLC-only cells are not the sole mechanism of raised sFLCs because levels of involved light chains >600 mg/l occurred in the serum of 6/32 (19%) of the M-Ig samples (compared to 10/16 (62%) for the dual group and 4/5 (80%) for the FLC-only patients).

There was no correlation between the percentage of the various cell populations and the serum levels of M-Ig or FLCs. This results from the large variation between individual tumours in absolute and relative M-Ig and FLC production as well as the variable quality and infiltration of bone marrow aspirates.

Dual populations are an example of intra-clonal heterogeneity in monoclonal gammopathies with implications for the biology of disease. The detection of M-Ig producing cells in the marrow of four free light chain disease patients provides evidence for their derivation from multiple myelomas making intact M-Ig in the natural history of the disease in such patients. Serological evidence of both light chain escape and intact immunoglobulin escape has been reported.¹⁰ Light chain escape may precede frank relapse in multiple myeloma. An abnormal sFLC ratio is a risk factor for progression of MGUS to myeloma.^{3 6} The finding of FLC-only cells in bone marrow may therefore have prognostic significance. The adaption of this double immunofluorescence staining procedure to flow cytometry and sorting would facilitate further immunophenotypic and genetic investigation of these cell populations.

The presence of minor FLC-only cell populations in more than a third of the monoclonal gammopathies making intact M-Ig supports other evidence that light chain escape may be expected more frequently with longer patient survival.^{11 12}

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Short report

Contributors MA conceived and designed the study, acquired, analysed and interpreted the data, and was involved in the drafting, revision and the decision to submit the paper for publication. NS performed the statistical analysis, interpreted data and was involved in the drafting, revision and the decision to submit the paper for publication. JB and SS provided clinical input and were involved in the drafting, revision and the decision to submit the paper for publication.

Competing interests None declared.

Patient consent Obtained.

Ethics approval London—Surrey Borders Research Ethics Committee approved the study.

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