Cytology of myeloma cells

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SYNOPSIS A cytological, cytochemical, and cytometric study of plasma cells from 195 cases of multiple myeloma showed that, contrary to earlier reports, flaming cells, thesaurocytes, and intranuclear inclusions are not confined to IgA-secreting cases but are common also in IgG and Bence-Jones varieties of myeloma. IgA-secreting cells are not larger, nor do they have a lower nuclear-cytoplasmic ratio than other myeloma cells. On average, for a given mass of tumour, Bence-Jones IgG, and IgA varieties of myeloma produce amounts of paraprotein in the ratio 1 to 1.6 to 2.7.

In 1961 Paraskevas *et al* reported a correlation between the morphological features of plasma cells in myeloma and the type of immunoglobulin secreted. The cases studied included 12 with γ_1A (β_2A , IgA) myeloma, 30 with γ (IgG) myeloma, and six myelomas without M protein (probably Bence Jones myelomas). Flaming cells, thesaurocytes, and intranuclear, PAS-positive inclusion bodies were found only in cases of IgA myeloma, and flaming cells especially were present in most cases and in high percentage in several. Cytometric studies showed a tendency for the plasma cells in IgA cases to have a larger diameter and smaller nucleus than those in IgG cases, and although these differences were individually small, their combination in a derived nuclear-cytoplasmic ratio showed a significantly lower ratio in IgA cases.

Some confirmation of these studies was provided by Maldonado *et al* (1965), who found conspicuous flaming cells in three of four patients with IgA myeloma but none in 15 patients with non-IgA myelomas. Brittin *et al* (1963) found examples of intranuclear inclusions in all varieties of myeloma and also in macroglobulinaemia, although PAS positivity of the inclusions occurred only in the cases of IgA myeloma and macroglobulinaemia. These authors also confirmed the impression that plasma cells of IgA myeloma were in general larger than those of non-IgA myelomas and more often showed cytoplasmic PAS positivity.

The continued prominence given to these reports in current textbooks of haematology (Williams *et al*, 1972; Wintrobe *et al*, 1974) leads us to describe here

the results of a larger scale survey carried out some years ago but previously unpublished.

Material and methods

The study was performed on bone marrow smears from 200 consecutive patients newly entered into a comparative trial of treatments in myeloma, under the auspices of the Medical Research Council. Five patients were subsequently excluded as not confirmed to have the disease, leaving 195 patients included in the survey. Cytological studies were carried out in ignorance of the immunoglobulin patterns and clinical state.

Smears stained with May-Grünwald-Giemsa were used to record the percentage of plasma cells present (a differential count of at least 400 cells was made in each case), the degree of cytoplasmic basophilia, the pattern of nuclear chromatin, the number of nucleoli, the predominant cell shape, the percentage of cells showing fragmentation and budding of the cytoplasm, and the percentages of polyploid cells, flaming cells, thesaurocytes, cells with nuclear inclusions, cells with Russell bodies, and Mott cells.

For the first 100 cases a detailed cytometric study was made, with measurements of the major and minor axes and the nuclear diameter of at least 100 plasma cells from each case, using a calibrated microscope eyepiece.

Cytochemical staining with the periodic acid-Schiff (PAS) reaction and with methods for acid and alkaline phosphatases was performed on those cases from which additional slides were available, and a total score for each reaction was determined by individually scoring 100 neutrophils (for alkaline

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phosphatase) and 100 plasma cells (for PAS and acid phosphatase) from each case according to a scale ranging from 0 to + + + + (as previously described for PAS and alkaline phosphatase by Hayhoe *et al* (1964)).

All the cytological features referred to above have been extensively described and illustrated in the past (eg, Paraskevas *et al*, 1961; Hayhoe and Flemans, 1969) and need no further definition or illustration here. The cytochemical techniques used were standard ones, as described by Hayhoe *et al* (1964) and Hayhoe and Flemans (1969).

After the cytological study had been completed information was obtained as to the type of immunoglobulin being produced in each case, and an assessment of the approximate daily production of M protein, derived from the serum level at presentation, the half-life of the M protein in question, and the extent of urinary loss of Bence Jones protein. The biochemical and immunoelectrophoretic studies and the calculations based on them were carried out by Professor J. R. Hobbs, who kindly made these data available to us and who has published elsewhere accounts of the technical methods used (Hobbs, 1967, 1969, 1971).

Results

The findings from this study can be most succinctly presented in a series of figures and tables.

DISTRIBUTION OF CASES

Table I shows the distribution of the 195 cases according to the type of immunoglobulin produced. The two examples of IgD myeloma, the four biclonal cases, and the three with no demonstrable M protein are excluded from the subsequent analyses.

CYTOMETRIC ANALYSIS

Figure 1 shows the mean cell diameters, major and minor, and the mean nuclear diameter in each of the three common immunoelectrophoretically separable

M protein	Total	Light chains		No L chains detected
		K	L	
IgG	107	75	25	7
IgA	44	25	17	2
IgD	2	—	2	—
BJ	35	21	14	—
Biclonal	4	{ AL, GK GK, L GL, ML GL, K		
No M protein identified: (but undoubted myelomas)		3		

Table I Multiple myelomas; immunoelectrophoretic analysis of 195 cases

Fig 1 Cytometry of myeloma cells associated with different types of paraprotein.

classes of myeloma, IgG, IgA, and Bence-Jones (BJ), further divided in each case according to the nature of the light chains, whether K (κ) or L (λ). The full data on 100 cases, amounting to some 30 000 individual measurements, were initially analysed by us without finding any significant differences in cell size or in nuclear-cytoplasmic ratio between the different biochemical classes of myeloma, and a subsequent computer-assisted analysis kindly performed by Dr R. G. Carpenter confirmed that no differences of statistical significance were present. Cells from IgA myelomas are not, then, significantly larger, nor do they have a lower nuclear-cytoplasmic ratio, than cells from IgG or BJ myelomas.

MARROW PLASMACYTOSIS AND PARAPROTEIN PRODUCTION

Figures 2, 3, and 4 are scattergrams showing the relationship between the percentage of plasma cells present in the initial marrow samples and the calculated daily production of paraprotein for each of the three large categories of myeloma, IgG, IgA, and BJ. The figures suggest that very little correlation exists between the two variables in individual cases. The percentage of plasma cells found in a marrow sample may vary widely according to the site sampled, since the disease tends to be unevenly

Fig 2

Fig 3

Fig 4

Figs 2, 3, and 4 Association between approximate daily production of 'M' protein and percentage of plasma cells in marrow in myelomas type IgG, IgA, and Bence-Jones respectively.

distributed, as radiological and postmortem studies clearly show, and perhaps the most to be made of these data comes from a consideration of average figures. For the IgG cases the mean percentage of plasma cells in this series was 31, and the mean daily paraprotein production was 13.7 g/70 kg; for IgA cases the respective figures were 33 and 24.8, and for BJ myeloma 39 and 11.1. If a generalization is to be made, though the wide scatter of individual figures detracts from its value, one might conclude that patients with BJ myeloma tend on average to have a higher burden of tumour cells at presentation (around 1250 g) than do IgG and IgA myeloma patients, who have around 1000 g, and that BJ, IgG, and IgA myeloma cells respectively produce amounts of paraprotein in a ratio of 1:1.6:2.7 for a given mass of tumour.

FLAMING CELLS

Figure 5 shows the percentage of cases in which flaming cells were recorded as present among each of the different varieties of myeloma, and figure 6 gives for each of these positive cases the percentage of plasma cells showing the flaming appearance. Clearly, the phenomenon is not restricted to IgA myelomas, although it is more common among them. When flaming cells are present they are as likely to be numerous in IgG and BJ myelomas as in IgA cases; the most striking example in this survey, with nearly 60% of the plasma cells showing flaming, was in a BJ myeloma.

Fig 5 Percentage of cases showing flaming cells in the different varieties of myeloma.

FRAGMENTATION AND CYTOPLASMIC BUDDING

Figure 7 shows the percentage of cases in each group of myelomas with >10% of plasma cells fragmenting or showing gross cytoplasmic budding. The feature is common but not significantly different in incidence in the three groups.

Fig 6 Percentage of plasma cells showing the flaming appearance in each positive case.

Fig 7 Percentage of cases in each myeloma group showing fragmentation or budding.

CYTOPLASMIC BASOPHILIA

This characteristic is prominent in the plasma cells from many cases of myeloma, being found, as figure 8 shows, in about 50% of all marrow specimens, but again there are no significant differences in its frequency among the separated groups. The mean daily paraprotein production in cases showing cytoplasmic basophilia was 16.1 g, and that for cases without basophilia was 15.5 g.

POLYPLOIDY

Figure 9 shows that plasma cell polyploidy was found more frequently in BJ and IgA myelomas than in IgG myelomas, but the incidence is common and the differences not great. When polyploidy occurred the multinucleated cells generally made up between 5 and 15% of all plasma cells, with occasional higher figures but no real differences among the immunoglobulin classes (fig 10).

THESAURACYTES, NUCLEAR INCLUSIONS, RUSSELL BODIES, AND MOTT CELLS

The percentages of cases showing these features are shown in table II. Thesaurocytes and Mott cells appear to be more common in IgA myelomas than in the other groups, whereas nuclear inclusions and isolated Russell bodies probably do not differ significantly in their frequency. The finding of single Russell bodies in 9 of 14 cases of BJ myelomas with L light chain production gives an unusually high

Fig 8 Percentage of cases with cytoplasmic basophilia +.

Fig 9 Percentage of cases showing >5% polyploid cells.

Fig 10 Percentage of polyploid cells in positive cases.

percentage incidence, but the number of cases is small and the difference is of doubtful significance. The table makes clear that none of these features can be regarded as in any way pathognomonic of a subvariety of myeloma.

CYTOCHEMICAL REACTIONS

The leucocyte alkaline phosphatase (LAP) scores, carried out on marrow neutrophils, are given in figure 11. Scores were frequently above the normal

% cases showing	IgG			IgA			BJ		
	K	L	All	K	L	All	K	L	All
Thesaurocytes	10	13	11	29	12	23	11	8	10
Nuclear inclusions	18	13	17	21	25	23	17	15	16
Russell bodies	27	36	29	29	19	25	17	62	36
Mott cells	13	—	10	25	25	25	11	15	13

Table II Incidence of thesaurocytes, nuclear inclusions, Russell bodies, and Mott cells

range (15 to 100), but their distribution did not differ significantly among the groups.

The PAS reaction rarely shows more than weak positivity in normal plasma cells, and the same proved to be true for plasma cells in myeloma. For illustrative purposes, it seemed best to divide the types of positivity encountered into three groups—one with nearly all cells negative, a second with predominance of weak diffuse cytoplasmic staining and occasional fine granularity, and the third with weak cytoplasmic tingeing but more frequent granularity. The distinctions are certainly ill-defined, with a subjective element in assessment, but give a broad indication of the degree of positivity. Figure 12 shows that about 50% of all myelomas in the survey had substantially PAS-negative plasma cells, the remainder having weak positivity (+), except for about 10% of both IgG and BJ cases, which showed

Fig 12 Percentage of PAS-positive plasma cells in all myelomas studied.

Fig 11 Distribution of leucocyte alkaline phosphatase scores in marrow neutrophils.

rather more marked positivity (++). None of the IgA cases showed more than weak (+) positivity.

Plasma cells generally show strong acid phosphatase positivity, and the intensity of reaction in individual cells can easily be graded on a 0 to ++++ scale. Total scores for each case studied are shown in figure 13. The strong reactions typical of normal plasma cells occur also in the plasma cells of myeloma, with average scores around 250, but no differences emerged between the immunoglobulin varieties.

Discussion

Immunoglobulin molecules have a basically similar structure, with four polypeptide chains, two heavy and two light, together with a carbohydrate moiety attached chiefly to the heavy chains. The different classes of immunoglobulin (IgG, IgA, IgM, IgD, and IgE) are distinguished primarily by the immunochemical specificity of their heavy chains but differ also in their tendency to polymerize and in their content of added carbohydrate. Thus IgG molecules show little polymerization and have about 3% carbohydrate, whereas IgA molecules polymerize more readily to 2, 3 or 4 unit structures and contain about 7.5% carbohydrate.

Fig 13 Total scores of acid phosphatase positivity in plasma cells for each case studied.

These chemical differences have been invoked to explain earlier reports of cytological features thought to be peculiar to the cells of IgA-secreting myelomas, namely, low nuclear-cytoplasmic ratio and frequent formation of flaming cells, thesaurocytes, and intranuclear inclusions. The present study shows that these features, far from being peculiar to IgA myeloma, are common in myelomas of all types, although flaming cells and thesaurocytes occur in a higher proportion of IgA cases.

Average figures for the percentage of plasma cells in the bone marrow at presentation and for the estimated daily paraprotein production show quite striking differences in the weight of paraprotein produced in the three classes of BJ, IgG, and IgA myelomas. For a given mass of tumour the output of paraprotein appears, on average, to be in the ratio of 1:1.6:2.7 for the three classes respec-

tively. The very wide scatter of figures from individual cases, shown in figs 2, 3, and 4, may be partly explicable on the grounds of the sampling variability inevitably associated with marrow aspiration but suggests also that gross functional abnormalities, with defective, unbalanced or excessive polypeptide chain function, must be a common feature in all types of myeloma.

Reference has not been made in this report to the very striking intracytoplasmic crystals, coarse azurophilic granules, and pseudo-Auer rods occasionally seen in plasma cells. Examples of these inclusions have been seen in each of the main varieties of myeloma in the present series, but the numbers are too few to provide a useful analysis of frequency in relation to the myeloma types.

Finally, we should emphasize that examples of each individual cytological feature discussed above, from flaming cells and thesaurocytes to polyploidy, Russell bodies, Mott cells and crystal formation, have been encountered in the marrow plasma cells from non-myelomatous states. They may be found, though uncommonly, in otherwise apparently normal marrow, and not infrequently in conditions associated with reactive plasmacytosis, such as rheumatoid arthritis, Hodgkin's disease, and disseminated carcinoma.

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