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PAPER

STEREOLOGICAL STUDIES ON MITOCHONDRIA AND ROUGH ENDOPLASMIC RETICULUM IN MYELOMA CELLS

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ABSTRACT

Stereological techniques were used to evaluate quantitative differences between plasma cells from immunologically different myelomas. Electron micrographs of plasma cell sections in bone marrow for ten patients were studied and thirteen parameters were estimated. No clear correlation was found between type of myeloma, level of paraprotein and quantitative ultrastructure.

INTRODUCTION

In multiple myeloma, malignant antibody-producing plasma cells infiltrate the bone marrow. These myeloma cells, which exhibit a wide range of ultrastructure (Blom, 1980), usually secrete a protein which can be detected in the serum and/or the urine of the patient. The disease is classified according to the immunological characteristics of the paraprotein. Although many previous investigators (Fisher and Zawadski, 1970; Ghadially, 1980; Reed et al, 1981) have looked for a correlation between cell morphology and the type of myeloma, these studies have been mainly qualitative and have yielded conflicting results. In this study which is still in progress, stereological techniques are used to examine this problem. The relationship between cellular structure and the serum level of paraprotein is also assessed. Myeloma cells are particularly suitable for this type of analysis because they are

usually derived from a single clone (Mellstedt et al, 1982).

MATERIALS AND METHODS

Bone marrow aspirates from patients with the following types of myeloma have been studied:- 5 with IgG kappa, 1 with IgG lambda, 2 with light chain kappa, 1 with IgA and 1 with non-secretory. The patients with non-secretory and IgA myeloma had received chemotherapy.

The bone marrow samples are fixed in 1.5% glutaraldehyde in 0.1 M sodium cacodylate for 3 hours at room temperature and washed in 7% sucrose sodium cacodylate buffer. The samples are post-fixed in osmium tetroxide, dehydrated in graded alcohols and embedded in Araldite. In each sample, sections are cut from five or six tissue blocks, and stained with uranyl acetate and lead citrate. From gold sections, a series of approximately 30 micrographs of randomly selected, nuclear-biased myeloma cell sections are taken at a final print magnification of 12000x. A second series of micrographs - at a final print magnification of 34000 x - are taken from silver sections. With each micrograph series, a calibration of the magnification is made using a cross-grating replica. Myeloma cells are identified on the basis that they contain substantial amounts of rough endoplasmic reticulum and do not show the typical features of the erythrocytic, phagocytic or granulocytic series (Bessis, 1973). The possibility that an occasional normal plasma cell may have been contained in the series for analysis cannot be excluded.

The stereological analyses are based on the following morphological model of a myeloma cell:-

1) All the cells are derived from a single clone.
2) The single spherical nucleus lies eccentrically in a spherical cell.
3) The rough endoplasmic reticulum (RER) compartment comprises the membrane system with attached ribosomes; it also includes the outer nuclear membrane. For volume fraction measurements, the cisternae, membrane and ribosomes are counted (Mayhew and Williams, 1974).

Volume fractions in the cell of the rough endoplasmic

reticulum (V_V RER) and mitochondria (V_V mit) are determined at magnifications of 34000 x and 12000 x respectively using a 0.5 cm square lattice grid and normal point-counting procedures. The surface area: volume ratio of the mitochondria (S/V mit) is calculated at the same time as V_V mit.

Cell volume is estimated from point-counting data (James, 1980) at a magnification of 1200 x. Hence the volumes per cell of the RER and mitochondria, and the surface area of the mitochondrial outer membrane (MOM) can be derived.

The surface area density (S_V) of the RER (per μm^3 of cytoplasm) and of the mitochondrial inner membrane (MIM) (per μm^3 of mitochondrial volume) are measured using a 1 cm^2 quadratic test lattice. The number of intersections with test organelle profiles per unit length of test line (P_L) were determined and S_V obtained from the relationship $S_V = 2P_L$ (Underwood, 1970). From these parameters the absolute surface areas per cell and the ratio of the two mitochondrial membranes can be derived.

An uncorrected estimate of the numerical density of ribosomes per unit area of RER membrane (Ns) is obtained by counting the total number of ribosomes (N) in the test area and dividing by the total RER surface area.

An estimate of the latter is obtained from a knowledge of total RER profile length (L) and section thickness (t) estimated from interference colours. $N_S = \frac{N}{txL}\ \mu m^2$

Further studies are in progress to measure N_S using the alternative relationship $\frac{N_V}{S_V}$ for comparison.

For ethical reasons, only a single sample is available from each patient and possible sampling variations cannot be discounted. Reasonably consistent results have been however obtained when micrographs of different cells from the same material have been analysed.

RESULTS

The results of the seven parameters of the mitochondria

and cell volumes for ten patients are shown in Table 1.

The parameters relating to the rough endoplasmic reticulum are seen in Table 2.

Figure 1 compares the values of the surface area of the RER per cell with the serum paraprotein level. The mitochondrial volumes per cell for each patient are seen in Figure 2.

DISCUSSION

The aim of the study is to see whether quantitative structural differences exist between the plasma cells from immunologically different myelomas. The results to date give no firm indication of a correlation between myeloma cell morphology and either the paraprotein type or level, thus being in agreement with earlier conclusions based on qualitative studies of Fisher and Zawadski (1970) and Ghadially (1980). However, positive relationships may emerge when a larger sample has been analysed. The striking feature about the results is the wide range of values between patients (see Fig 1,2). As far as it has been possible to ascertain, these differences are independent of sampling variations.

Factors which influence the level of paraprotein in a patient's serum are the tumour mass (Nathans et al, 1958), the catabolic breakdown of M-protein (Salmon and Smith, 1970) and the production and secretion rates of the individual cell (Salmon and Smith, 1970). This latter factor, one could postulate, should be affected by the number of ribosomes per cell and the surface area of the RER per cell. In Fig 1, the RER surface area and paraprotein level are compared. The highest values for both parameters appear in one patient (ED) and so do the lowest values (DH). However, on statistical analysis no significant correlation was detected between paraprotein level and RER surface area studied here. Similarly, no general correlation was demonstrated between myeloma type and the other cell parameters studied (see Fig 2 and tables 1,2). However, the patient DH with non-secretory myeloma, whose cells were shown by immunofluorescent studies to be non-producing as well, had the lowest values for some RER parameters (S_V RER, surface area of RER, N_A and number of

Table 1. Paraprotein type and level, and parameters relating to the mitochondria

Patient	Ig type	Ig level (g/l)	V_v mit. %	S/V mit. (μm^{-1})	mit. volume per cell (μm^3)	Mit. inner membrane: Surface area density (μm^{-1})	Mit. inner membrane: Surface area per cell (μm^{-2})	Mit. outer membrane: Surface area per cell (μm^2)	$\frac{\text{MIM SA}}{\text{MOM SA}}$	Cell size (μm^3)
DJ	IgG k	81.0	4.5	7.3	12.6	20.0	250	92	2.75	281
MK	IgG k	-	4.6	6.6	24.6	22.4	550	162	3.40	536
ED	IgG k	100.0	6.3	8.5	47.6	26.5	1260	403	3.13	755
MA	IgG k	9.1	3.6	7.3	16.1	20.5	330	118	2.80	446
RW	IgG k	17.0	8.3	9.5	35.7	22.6	810	340	2.38	430
ER	light chain k	-	9.8	7.3	71.0	24.6	1750	518	3.37	724
HO	light chain k	-	6.0	8.8	17.2	18.0	310	151	2.05	287
HB	IgA	19.9	3.6	8.5	11.1	23.6	260	95	2.76	308
KB	IgG 1	-	3.8	8.6	14.6	-	-	126	-	384
DH	Non-secretory	0	3.7	8.3	10.6	22.7	240	88	2.74	286

Table 2 Parameters relating to the Rough Endoplasmic Recticulum

Patient	Surface area density of RER (μm^{-1})	Surface area of RER per cell (μm^2)	V_V RER %	Volume per cell (μm^3)	N_A ribosomes (μm^{-2})	No. of ribosomes per cell ($x10^5$)
DJ	5.8	1050	12.0	33.7	130	1.4
MK	7.0	2570	15.9	85.1	120	3.1
ED	4.6	2650	21.9	165.1	-	-
MA	5.9	1880	14.8	66.0	110	2.1
RW	4.3	1210	8.0	34.4	-	-
ER	5.0	2890	12.9	93.4	-	-
HO	5.1	1040	10.6	30.4	121	1.3
HB	5.7	1210	11.6	35.7	138	1.7
KB	6.4	1750	-	-	-	-
DH	3.9	760	14.2	40.7	88	0.7

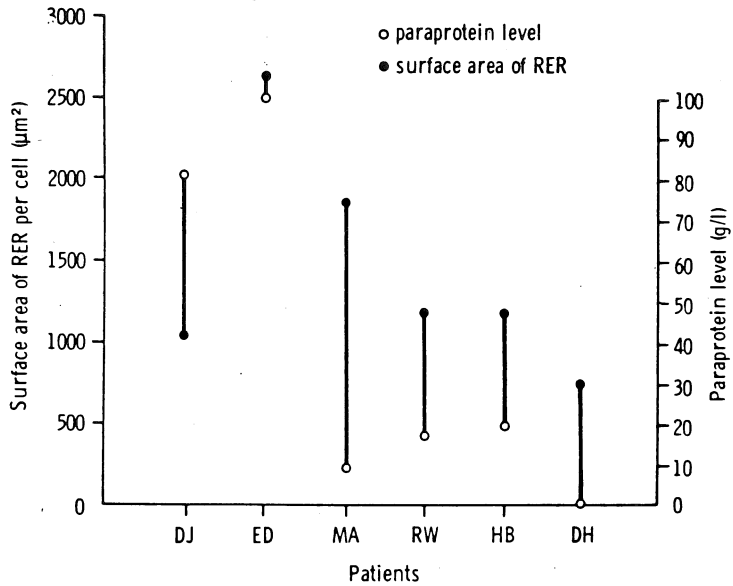

Fig.1. Paraprotein level and surface area of RER per cell

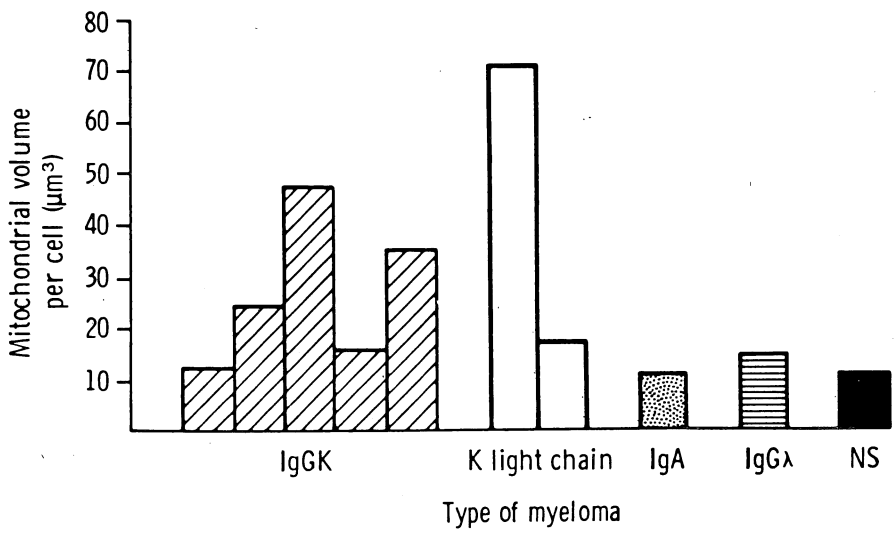

Fig.2. Mitochondrial volume per cell and type of myeloma

ribosomes per cell). Mancilla and Davis (1977) mention a case of non-producing myeloma in which the RER in the cells was poorly developed, and Azar et al (1972) describes cells in non-secretory myeloma where the RER contained 'skip' areas where no ribosomes were present but this was also seen in reactive plasma cells. The parameters for the RER will be underestimated (Mayhew and Williams, 1974) because the membrane is often sectioned tangentially and is not clearly visible for measurement.

Stereological studies on other single cell populations have given results which provide a useful comparison with those obtained here. For example, normal macrophages (Mayhew and Williams, 1974) have a V_V RER of 2.8%, reflecting the fact that macrophages contain smaller amounts of RER than plasma cells. On the other hand, small lymphocytes in chronic lymphocytic leukaemia (also a B-cell neoplasm) have a mitochondrial volume fraction of 3.4% (James, 1980), which is comparable to the lower values obtained here.

In conclusion, stereological values for human myeloma cells are presented for the first time. Wide variations have been found between different patients, but no evidence has so far emerged that cells producing different types and amounts of paraprotein show any corresponding differences in the structures most closely involved in protein production.

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