Quantitative study of the immunoglobulin-containing cells in trephine samples of bone marrow

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SUMMARY Immunoglobulin (Ig) was demonstrated in paraffin sections of 12 trephine bone marrow biopsies by means of the unlabelled antibody peroxidase-antiperoxidase (PAP) method. The Ig-containing cells, which were counted with the Reichert-Jung (Kontron) MOP-AMO₃ user-controlled image-analyser, were found to constitute approximately 4.2% of all the nucleated cells in the marrow, a figure significantly higher than those reported by previous workers.

Estimates of the numbers of plasma cell numbers in bone marrow have generally been made on aspiration biopsies^{1,2} and have been based on morphological criteria. One previous study made use of disaggregated aspirated marrow specimens with immunofluorescent demonstration of immunoglobulins.³ In the present study, plasma cells were detected by demonstrating immunoglobulin (Ig) within their cytoplasm by means of the unlabelled antibody peroxidase-antiperoxidase (PAP) method. Moreover, instead of aspiration biopsies, trephine samples were used. Eleven of the trephine samples were from patients being investigated for the extent of their malignancy and one was from a case of pyrexia of unknown origin. None showed histological evidence of neoplastic infiltration of the marrow and the haemopoietic tissue was reported as morphologically normal in all the specimens. Cell counting was greatly simplified by the use of the Reichert-Jung (Kontron) MOP-AMO₃ operator-controlled image analyser.

Material and methods

Twelve specimens of marrow were taken from the posterior iliac crest by means of a Jamshidi needle from the same number of patients. Six patients had nodal Hodgkin's disease, two had nodal non-Hodgkin's disease, three had bronchial carcinoma and one had pyrexia of unknown origin.

FIXATION AND PROCESSING

Ten specimens were fixed for 1.5 h at 20°C in formol sublimate and two specimens for 24 h in formol

saline containing 2% acetic acid. After this period of preliminary fixation, all specimens were fixed for two days at 20°C in formol saline solution to which 10% glacial acetic acid had been added. The latter solution also acted as a decalcifying agent.⁴ After fixation the specimens were dehydrated through graded ethanols, cleared in chloroform and embedded in Ralwax (RA Lamb). The high polymethyl content of Ralwax makes it particularly suitable for trephine specimens. Sections were cut at 3 µm.

PAP METHOD

Immunoglobulins (γ, α and μ heavy chains; κ and λ light chains*) were demonstrated by the PAP method.⁵ Trypsinisation was not used, being unnecessary with acetic acid-containing fixatives.⁴ The optimal dilution of the specific antiserum against light or heavy chain was 1/500 or 1/1000.

CONTROLS

Sections were exposed to DAB-hydrogen peroxide alone; normal rabbit serum was substituted for the specific antiserum to light or heavy chain; and a range of dilutions of IgG, IgA and IgM was added to each specific antiserum to block the reaction to each heavy chain.

COUNTING PROCEDURE

The Reichert-Jung (Kontron) MOP-AMO₃ image-analyser employs a sensitive light-emitting pen to count (or measure the area or maximum diameter of) objects seen superimposed on the pen-tip by means of a conventional camera lucida drawing tube. The information thus obtained is fed to the programmed

*Dakopatts 10-090, 10-MAT, 10-091, 10-9K₂, 10-9L2, 21-090, Z113 (Mercia Brocades Ltd, Weybridge, Surrey).

microprocessor and stored on one of the 20 channels available in the machine. At least 1000 nucleated marrow cells were counted in each PAP preparation and stored on one channel. For each field examined, the number of heavy or light chain-containing cells counted was stored and summated on another channel. At the end of this procedure, the machine printed out the totals on the two channels and expressed the number of heavy or light chain-containing cells as a percentage of the total nucleated cell count for the fields examined. The number of cells containing each heavy or light chain was estimated separately. In this way the total percentage of cells containing a heavy chain (γ , α and μ) and the total percentage of cells containing a light chain (κ or λ) were obtained. The ratio of the number of κ -containing cells to λ -containing cells was also calculated.

Results

Apart from the occasional megakaryocytes which gave a weak positive cytoplasmic reaction for Ig (γ , α , μ , κ and λ chains) and which were not included in the count, the Ig-containing cells appeared to be plasma cells of varying maturity. The number of such cells containing the various types of heavy or light chain are shown in the Figure, expressed as a

Number of cells containing various types of heavy or light chain as a percentage of total numbers of nucleated cells. Cross bars represent mean values.

Numbers of cells containing a heavy or light chain and the mean κ : λ ratio

	% of cells containing:					Total $\gamma + \alpha + \mu$	Total $\kappa + \lambda$	Ratio κ : λ
	γ	α	μ	κ	λ			
Mean value	2.34	1.28	0.55	2.68	1.5	4.17	4.18	1.78
	(56)	(31)	(13)	(64)	(36)			
Standard error of mean	0.12	0.09	0.04	0.08	0.04	0.13	0.10	0.06

Figures in brackets represent the % of heavy and light chain-containing cells related to the total γ , α or μ chain-containing cells or κ or λ chain-containing cells.

percentage of the total number of nucleated cells. The number of cells containing each type of chain is seen to vary only slightly from specimen to specimen. The Table shows the mean number of cells containing a heavy or light chain, expressed as a percentage of the total number of nucleated cells. The total percentage of cells containing heavy chains (4.17%) is close to the total percentage of cells containing light chains (4.18%), and the mean ratio of the κ chain-containing cells to the number of λ chain-containing cells is 1.78. The Table also shows the number of cells containing a particular heavy chain (γ , α or μ) expressed as a percentage of all the cells containing a heavy chain (γ plus α plus μ); thus, cells containing γ chain constitute 56% of all heavy chain-containing cells, those with α chain 31%, and with μ chain 13%. Similarly, cells containing κ chain form 64% of all light chain-containing cells, λ chains being present in the other 36%.

Discussion

Bone marrow trephine specimens are frequently taken for histological examination, as an adjunct to smear preparations, especially as part of a staging procedure for Hodgkin's disease and non-Hodgkin's lymphomas. Eleven of the 12 specimens used in the present study were taken as part of a staging procedure and all were reported as negative—that is, the haemopoietic tissue was histologically normal in all respects. The acetic acid-containing fixatives used effectively decalcified the sections and readily allowed the preparation of thin (2-4 μ m) flat paraffin sections which reacted well with the PAP method without prior trypsinisation. Cell morphology was also good, thereby facilitating the counting of cells on the Kontron MOP-AMO₃ image analyser.

Quantification is being increasingly used in histopathology and automatic or semiautomatic measurements have been made on a variety of tissues, including lung,⁶ muscle⁷ and jejunal mucosa;⁸ lymph node preparations have also been studied.⁹ The techniques now available are fast and accurate and the image analyser used in this study made the counting of large numbers of cells relatively easy.

Our finding of Ig in the cytoplasm of some mega-

karyocytes was unexpected. However, as with the plasma cells, the reaction was blocked by the addition of the appropriate heavy chain antigen to the specific antibody used in the first stage of the PAP sequence, confirming that Ig in the cytoplasm was responsible and not some contaminating anti-megakaryocyte antibody in the reagent. Megakaryocytes do not synthesise Ig and presumably the Ig-positive cells absorbed immunoglobulin from the environment. Their presence did not interfere in any way with the counting of the Ig-containing cells of the plasma cell series.

In one published series, based on a morphological study of smears of bone marrow aspirates from 12 healthy men, plasma cells were found to constitute 1.3% of all the nucleated marrow cells,¹ and an upper limit of 2.3% is generally accepted.² However, Turesson³ used immunofluorescence to detect cells containing Ig in dispersed bone marrow preparations, obtaining a value of 3.01% for Ig-containing cells. The $\kappa:\lambda$ ratio was found to be 1.5 and the numbers of IgG-containing cells exceeded that for IgA-containing cells, with cells containing IgM being the smallest in number. Our figure for the $\kappa:\lambda$ ratio (1.78) is higher than that found by Turesson (1.5). However, our percentages of cells containing γ , α and μ heavy chains closely resemble Turesson's figures; whereas our data show γ chain-containing cells to constitute 56% of the heavy chain-containing cells, α chain cells 31% and μ chain cells forming 13%, in Turesson's series, γ -chain containing cells were 55% of cells containing heavy chains, α chain formed 30% and μ chain 15%. We found Ig-containing cells to constitute a rather higher proportion of the nucleated cells in the marrow; thus the figure for $\kappa + \lambda$ light chain-containing cells was 4.18% (range 3.73-4.90), and for $\gamma + \alpha + \mu$ heavy chain-containing cells it was 4.17% (range 3.60-4.95). The higher figures which we obtained for some cells may have resulted from the use of the sensitive PAP technique, better fixation or from the use of iliac crest trephines instead of sternal marrow aspirates. On the other hand it has been shown that the number of plasma cells is often increased in the bone marrow of patients with malignant disease.² This could explain the larger numbers of Ig-containing cells in 11 of our preparations, but the fact that the total

numbers of Ig-containing cells was more or less constant from patient to patient, irrespective of the type of malignancy, makes it unlikely. Certainly, if malignancy had any effect on the marrows, it must have been non-specific. However, the presence of malignant cells actually within the marrow might reasonably be expected to influence the number of Ig-containing cells there. We now propose to count the Ig-containing cells in marrows infiltrated by malignant cells, using the results of the present study as controls.

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