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THE DIFFERENTIATION OF MYELOMATOSIS FROM OTHER CAUSES OF BONE MARROW PLASMACYTOSIS

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The proportion of plasma cells in aspirates of bone marrow is occasionally increased in conditions other than myelomatosis (Bing, 1940; Bayrd, 1948; Fadem and McBirnie, 1950; Fadem, 1952; Bessis, 1956). In cases of this sort (the so-called "plasmacytic response"), one may see immature cells, sometimes in clumps, cytoplasmic vacuolation, multinucleated cells, and degenerative changes of different kinds (Hayhoe and Smith, 1951). Paris and Bakke (1956) reported a case of agranulocytosis in which the marrow aspirate contained 60.2% of plasma cells. They observed that the occasional difficulty in distinguishing such plasmacytoses from myelomatosis is analogous to the difficulty in distinguishing a leukaemoid reaction from leukaemia. If, as may happen, myelomatosis cannot be excluded on clinical or other grounds a diagnostic impasse may result.

Fadem (1952) compared the marrow findings in 110 cases of plasmacytic response with those in 52 cases of myelomatosis and was able to establish a number of broad differences between the two groups. For example, the marrow in plasmacytic response was usually normally cellular, and the plasma cells were mainly mature, uniformly distributed, often related to reticulum cells, and rarely more numerous than 20% of the total count. In myelomatosis, on the other hand, the marrow was most often hypocellular, and the plasma cells mainly abnormal, shifted to the left, focal in distribution, rarely associated with reticulum cells, and usually more numerous than 20%.

It is possible to criticize such differential criteria in that they are general statistical trends which are true of large groups but afford only a low degree of probability in individual cases. Thus Paris and Bakke (1956) rely particularly on the higher proportion of proplasmacytes in myelomatosis, but they consider that borderline cases may occur in which doubt may remain as to the true nature of the process. A narrower and more objective criterion would, therefore, seem desirable.

MacCarty (1929, 1936) called attention to the enlarged nucleolus of the cancer cell and suggested its diagnostic value. Quensel (1928a, b, and c) examined the cells of pleural and peritoneal effusions using a supravital technique in which nucleoli were stained by methylene blue. The atypical cells of malignant effusions frequently showed nucleolus/nucleus ratios in the upper part of the range 0.20 to 0.66. The normal endothelial cells of the same fluids showed nucleolus/nucleus ratios of 0.12 to 0.20.

The nucleolus of the myeloma cell is always abnormal and often strikingly hyperplastic (Bessis, 1956). Its importance in diagnosis has been stressed by Streicher, Sandkühler, Roth, and Schwenkenbecher (1953). The mere observation of enlarged nucleoli may not always be sufficient for diagnosis, however, since plasma cells which are responding to antigenic stimulation may also show this feature. It will be suggested in this paper that assessment of the nucleolus/nucleus (n/N) ratio gives a more reliable morphological distinction between plasmacytic responses and myelomatosis.

Materials and Methods

Smears of marrow obtained by aspiration biopsy from 10 consecutive cases of myelomatosis were compared with those from 10 consecutive assorted cases in which the proportion of plasma cells was increased. The normal marrow plasma cell percentage was discussed by Hayhoe and Smith (1951), who concluded that 2% and more were pathological: their conclusions are adopted here. The relevant details of these cases are set out in the Tables. Nucleoli were demonstrated negatively by applying the Feulgen reaction for desoxyribonucleic acid as follows (Gardikas and Israels, 1948, though using routine methyl alcohol fixation): (1) Washed in tap-water for 10 minutes; (2) washed in distilled water for two minutes; (3) placed in N HCl (room temperature) for two minutes, then (4) in N HCl at 60° C. for eight to 10 minutes, then (5) rinsed in N HCl (room temperature); (6) rinsed in distilled water; (7) stained with leucobasic fuchsin (Schiff reagent) for one and

TABLE I
RELEVANT FEATURES OF 10 CASES OF MYELOMATOSIS

Case	Marrow Myeloma Cells (%)	Nucleolus/ Nucleus Ratio	Remarks
1	39	0·49	Multiple focal osteolytic deposits
2	11	0·47	,, ,, ,, ,,
3	60	0·46	Plasma cell leukaemia
4	12	0·43	Multiple focal osteolytic deposits
5	100	0·42	Severe osteoporosis; aspirate probably from focal deposit
6	23	0·40	Plasmacytoma of humerus; slow generalization of disease over following 3 years
7	37	0·39	Diffuse marrow involvement; presented as "primary" amyloidosis
8	21	0·39	Severe osteoporosis
9	50	0·34	Diffuse marrow involvement
10	11	0·32	Multiple focal osteolytic deposits; extensive amyloidosis

TABLE II
RELEVANT FEATURES OF 10 CASES OF PLASMACYTIC RESPONSE

Case	Final Diagnosis	Marrow Plasma Cells (%)	Nucleolus/ Nucleus Ratio	Remarks
1	Adenocarcinoma of colon	7·0	0·29	E.S.R. 137 mm./hr. Atypical plasma cells suspicious of myeloma. Plasma proteins, total 8·8 g., globulin 5·7 g.
2	Tabes dorsalis	13·0	0·29	Many atypical plasma cells, suspicious of myeloma
3	Carcinomatosis	6·0	0·27	
4	Aplastic anaemia	15·0	0·27	
5	Pulmonary tuberculosis	5·0	0·26	
6	Congenital syphilis; probable rheumatoid arthritis	3·6	0·25	E.S.R. 130 mm./hr. plasma proteins, total 7·3 g., globulin 4·9 g.
7	Cirrhosis of the liver	4·0	0·25	
8	Subacute nephritis; chronic rheumatic endocarditis	9·0	0·24	Many atypical plasma cells, suspicious of myeloma. Plasma proteins, total 7·1 g., globulin 4·2 g.
9	Aplastic anaemia	13·0	0·24	
10	Frontal astrocytoma; chronic liver disease	3·4	0·22	E.S.R. 120 mm./hr.

a half to two hours; (8) put through two or three changes of SO_2 water, each of one minute's duration; (9) washed in tap-water for 10 to 15 minutes; (10) dehydrated in graded alcohols, cleared in xylol, and mounted.

The SO_2 water was prepared from sodium metabisulphite by treatment with hydrochloric acid (5 ml. 10% $Na_2S_2O_5$, 5 ml. N HCl, water to 100 ml.).

A simpler modification of this method omitting stages 1, 3, and 5 was found quite satisfactory. Careful control of time and temperature during the period of hydrolysis (stage 4) is important in securing consistent results. Counterstaining is not essential but facilitates the recognition of plasma cells by showing their cytoplasm. To this end the smears may be immersed in 0.01% fast green FCF in 95% alcohol (Lillie, 1954) for a few seconds, or in Jenner's stain (Gardikas and Israels, 1948) for 10 minutes. Counterstaining follows stage 9 of the technique.

Nuclear and nucleolar diameters were measured with a Zeiss screw micrometer eyepiece mounted on a monocular microscope. All measurements were made in a darkened room, using the same instrument throughout. In order to minimize factors of variation such as thickness of smear, intensity of staining, or irregularity of nuclear and nucleolar outline, 25 cells were measured in each case. These were sought in as many random areas as were necessary. An average of the 25 n/N ratios was then taken. Degenerate cells and those with obscured nucleoli were ignored. Reticulum cells and the earlier normoblasts occasionally simulated the chromatin pattern of plasma cells; where doubt could not be resolved the cell in question was rejected. Many normal mature plasma cells were bypassed in cases of plasmacytic response because no clear nucleolus was discernible. Such cells were very rarely seen in cases of myelomatosis.

As the method of assessment was quantitative and objective, tests of reproducibility were not considered essential. A rough index of the attainable accuracy is given by the fact that in four cases (two from each group) remeasurement after a lapse of time yielded ratios agreeing with the originals to within 0.02.

Results

The average n/N ratios, calculated from the ratios of 25 random cells in each of 10 cases of plasmacytic response, ranged from 0.29 to 0.22 with a mean of 0.26. Similarly calculated in 10 cases of myelomatosis it ranged from 0.49 to 0.32, with a mean of 0.41. The boundary between the two categories is about 0.31 and there is no overlap. As nuclei and their nucleoli varied somewhat in size, especially in myelomatosis, it was considered that absolute measurements would vary too much to be useful and so were not calculated.

Though morphology was of secondary interest, certain features came to be recognized as typical of the myeloma cell nucleus. The nucleolus was usually central or slightly eccentric and there was a notably thick nucleolar membrane (Fig. 1). The nucleolar substance occasionally showed a curious bluish refractility. There was prominent nucleolus-associated chromatin in most instances. The specificity of the Feulgen reaction for D.N.A. enabled almost quantitative comparisons to be made between these nuclei and those of normal immature plasma cells in which the nucleolus, even when large, was always delicately bounded (Fig. 2). It also enabled an estimate to be made of the degree of maturity of individual cells. Normal

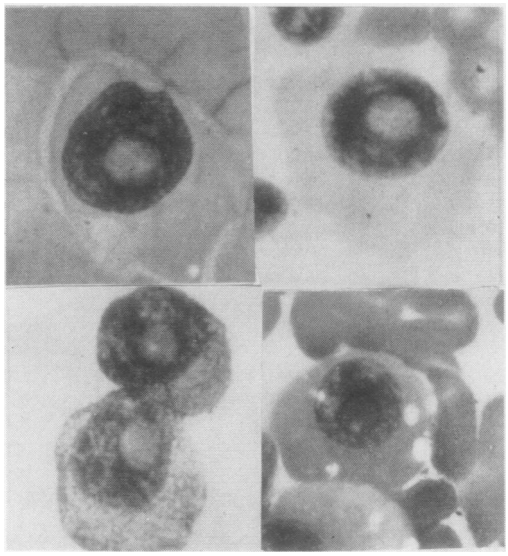

FIG. 1.—Typical myeloma plasma cells. Feulgen–Jenner, × 800.

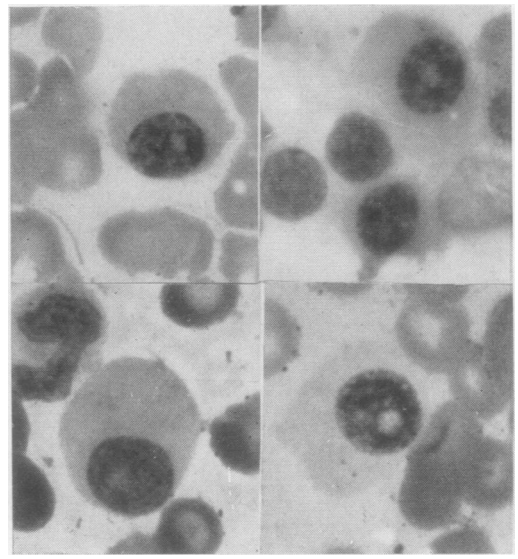

FIG. 2.—Plasma cells from four cases of plasmacytic response. Feulgen–Jenner, × 800.

mature Marschalko plasma cells were commonly seen in cases of plasmacytic response, but rarely, and doubtfully, in myelomatosis.

The Feulgen technique used (essentially that proposed by Gardikas and Israels (1948) for other haematological purposes) was found to be simpler, more delicate, and more controllable than the Unna–Pappenheim procedure. Romanowsky stains are unsuitable as they frequently obscure nucleoli completely.

Comment

The present study employs a rather small series of cases from which dogmatic conclusions may not be drawn. The results obtained by micrometry do, nevertheless, amply confirm the visual impression of greater nucleolar enlargement in myelomatosis than in other forms of marrow plasmacytosis. They suggest, moreover, that this difference in size, supplemented perhaps by differences in structure, may be of practical value in diagnosis.

There is a miscellany of diseases in which the proportion of marrow plasma cells is increased. In many of them an elevation of plasma globulins is found as well, possibly due to autoantigenic stimulation (Hayhoe and Smith, 1951). A clinical suspicion of myelomatosis may be raised, for example, by carcinomatosis, particularly when there are osteolytic secondary deposits (Marchal and Mallet, 1948, quoted by Bessis, 1956), by tuberculosis in some of its manifestations, by rheumatoid arthritis (Hayhoe and Smith, 1951), by anarthritic rheumatism (Bagratuni, 1956), by hypersensitivity states (Paris and Bakke, 1956), or by syphilis. The responsibility for diagnosis may then be felt to lie with the clinical haematologist. Case 1 of the plasmacytic response group illustrates this situation.

C. E. (Reg. No. 224913), a man aged 71, presented with a history of pain in the lower lumbar region, left loin, and left leg; he was anaemic (Hb 66%, 9.8 g.); his E.S.R. was 137 mm./hr.; his plasma proteins were raised to 8.8 g./100 ml., of which 5.7 g. were globulins; a marrow aspirate contained 7% of plasma cells, many of which were atypical. Post-mortem examination revealed an advanced adenocarcinoma of the sigmoid colon which was infiltrating the tissues of the left side of the pelvis. There was no evidence of myelomatosis.

In cases such as this, when even the marrow smears are equivocal, assessment of the n/N ratio may give a valuable clue to the correct diagnosis.

Summary

The ratio of nucleolus to nucleus in the plasma cells of 10 cases of myelomatosis was consistently greater than that in plasma cells of 10 cases of "reactive" bone marrow plasmacytosis (so-called plasmacytic response). It is suggested that this finding may be of practical diagnostic value in equivocal cases.

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