Marrow dysplasia with C trisomy and anomalies of the granulocyte nuclei

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SYNOPSIS An unusual case of bone marrow dysplasia is reported. The features of particular interest are the very high incidence of nuclear drumsticks on the polymorphs, a curious appearance of these same nuclei on electron microscopy, and C trisomy of the bone marrow cells; it is possible that the condition resulted from gold and/or x-ray treatment.

We have not found any cases reported similar to that presented here.

CASE REPORT

The patient was an 84-year-old woman who had for 19 years (up to January 1965) received various forms of treatment for 'arthritis', understood by her to be rheumatoid arthritis. The remedies included vaccines, adrenaline, progesterone, pyridoxin and other vitamins, all in innocuous doses. In 1959 she also had x-ray therapy to the lumbar spine (170 kv. $\frac{1}{2}$ Cu + 1 Al filter 35 c.mm. F.S.D. $150 \times 5 = 750$ total dose), intra-articular cortisone to each knee and some prednisolone. From 1960 onwards she was treated with injections of Auro-Calcium (total 1,025 mg.) changed in 1963 to Myocrisin (total 420 mg.).

In August 1964 she was noticed to be pale and was treated with parenteral iron and vitamins, folaemin, and hog's plastules. In October 1964 the haemoglobin was found to be 8.7 g.% and by January 1965 had fallen to 6.1 g.%. On admission then to the Royal Marsden Hospital the only further symptoms to be added were anorexia, loss of 1 stone in weight, and epigastric pain after food, all dating back for four to six weeks.

Examination revealed pallor, a smooth tongue, slight sacral oedema, and a few ecchymoses on the legs. Pulse was 92, regular; blood pressure 110/50 mm.Hg; spleen impalpable, liver just palpable. There was slight bony swelling of both knees and one Heberden's node on the left hand with slight wasting of the quadriceps and small muscles of both hands. However, there was no clinical evidence of rheumatoid arthritis either active or past, and radiographs showed only degenerative joint changes with osteoporosis. A barium meal showed no gastric abnormality.

LABORATORY INVESTIGATIONS Haemoglobin 5.4 g. per 100 ml. (37%); R.B.C.s 1.52 million per c.mm.; P.C.V. 15%; M.C.V. 99 cu. μ ; M.C.H. 35 μ g.; M.C.H.C. 36%; reticulocytes less than 1%; W.B.C.s 4,800 per c.mm.

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(polymorphs 60%, lymphocytes 15%, monocytes 13%, eosinophils 2%, metamyelocytes 6%, myelocytes 2%, blast cells 2%). Blood group was O Rh-positive. The blood urea level was 54 mg.%, serum bilirubin 0.5 mg.%, plasma sodium 138 mEq./l., potassium 4.2 mEq./l., chloride 104 mEq./l., and bicarbonate 23 mEq./l. Serum protein 5.8 g.% (albumin 2.3 g.%, globulin 3.5 g.%). Electrophoresis showed some reduction in albumin and diffuse increase of gamma globulin. Serum alkaline phosphatase was 8 King-Armstrong units, thymol turbidity 4 units, thymol flocculation positive, SGP transaminase 127 units; prothrombin concentration was 100%, serum iron 215 μ g. per 100 ml., iron binding-capacity 225 μ g. per 100 ml. (saturation 96%). Differential agglutination (Rose-Waaler) test negative; test for antinuclear factors negative. No L.E. cells found. Serum vitamin B₁₂ 430 μ g./ml. Serum folate (*L. casei*) 7.5 μ g./ml. Urine contained a trace of protein, very scanty casts and cells. Three specimens of stool tested for occult blood were negative. Bone marrow aspirated from the sternum was found to be cellular and composed predominantly of granulopoietic cells.

Differential count (200-5,000 cells)

Neutrophils (segmented)	19.0%
Neutrophils (band)	11.5%
Metamyelocytes	23.0%
Myelocytes	19.0%
Promyelocytes	6.0%
Eosinophils	0.5%
Eosinophil precursors	2.0%
Basophil precursors	1.0%
Monocytes and promonocytes	6.0%
Lymphocytes	9.0%
Other cells	3.0%

The last included:

Blasts and blast-like cells	33/5,000
Plasma cells	28/5,000
Late normoblasts	7/5,000
Probable early normoblasts	16/5,000
Number of mitoses in marrow	22/5,000

A few normal megakaryocytes could be found. The few late normoblasts were readily classified but showed minor anomalies such as irregular nuclei. The earlier erythropoietic cells were less easy to distinguish and could be confused with both plasma cells and blast cells.

GRANULOCYTE MORPHOLOGY The appearance of the mature granulocytes can be seen in Figs. 1-3. A high proportion of the segmented neutrophils and eosinophils display a well-formed pedunculated drumstick and the same is seen in some of the band neutrophils. Many of the drumsticks have a classical pear-drop shape but in others the shape is nearer to a circle or even to a triangle or diamond with blunted corners. In addition a certain number of non-pedunculated nuclear projections can be seen; the distinction between a drumstick and a projection cannot be made if the pedicle of the drumstick overlies the main mass of the nucleus. An analysis of 500 cells is given in Table I. A search of several buffy coat films covering 20-30,000 polymorphs has revealed a single cell (Fig. 3) with equivocal double drumsticks. Cells bearing one drumstick and one projection are fairly frequent (Figs. 1 and 2). In some films a proportion of the neutrophils have contained Dohle bodies. The leucocyte alkaline phosphatase reaction was negative on two occasions; P.A.S. and peroxidase staining were normal.

The early cells of the granulocyte series as seen in the bone marrow were morphologically normal and, indeed, the later stages of maturation, seen also in the blood (Figs. 1 and 2), would not normally have attracted attention. However, in so far as one can analyse the two components of nuclear maturation, namely chromatin condensation and nuclear segmentation, it seemed that the

TABLE I
ANALYSIS OF NUCLEAR PROJECTIONS IN 500 NEUTROPHILS
Number of Lobes

	<i>Unsegmented</i>	2	3	4-5	Total
Drumstick	25	88	86	18	217
Projection	15	53	45	7	120
Drumstick and projection	2	12	13	0	27
Two projections	1	3	1	1	6
No drumstick or projection	36	50	38	6	130
Total	79	206	183	32	500

segmentation process tended to be precocious (*cf.* Pelger anomaly where the opposite is the case).

ELECTRON MICROSCOPY For electron microscopy, buffy coat cells were fixed in 3% glutaraldehyde and post-fixed in 1% osmium tetroxide; they were embedded in araldite and sections stained in alkaline lead solutions. To obtain electron micrographs of nuclear drumsticks is difficult as the sections used are thin and the chance of obtaining a section showing a drumstick attached to the nucleus is small; moreover it is impossible from a single section to interpret such structures with certainty; ambiguity can only be avoided with serial sections. Nevertheless two nuclear modifications were seen in these cells. The first and most common is shown in Figs. 4 and 5; it is merely a nuclear protrusion and appears to be filled with normal nuclear chromatin. The other less common abnormality (Fig. 4) consists of an excess of nuclear membrane enfolding an area of cytoplasm; it is impossible to say

FIG. 1. *Buffy coat* $\times 1,600$.

FIG. 2. *Buffy coat* $\times 1,600$.

FIG. 3. *One polymorph in 20,000 screened. Two drumsticks?* $\times 4,000$.

whether such areas are completely surrounded by nuclear membrane material. There is no abnormality in the structure of the nuclear membrane itself. The cytoplasm of these cells is normal except perhaps for the occasional presence of small osmiophilic lamellar bodies (Fig. 5).

CHROMOSOME ANALYSIS The chromosomes of the bone marrow were examined on two occasions by a modification of the direct method of Tjio and Whang (1962). Karyotypes were prepared from a total of 20 cells, of which 16 had an extra chromosome in the C group (X-6-12). The details are given in Table II and a karyotype is demonstrated in Figure 6.

It is not possible to say whether the extra C chromosome

TABLE II				
CHROMOSOME STUDIES				
Date	Preparation	No. of Chromosomes	No. of Cells	Analysis
26.2.65	B.M. Direct	47	10	+1C
		47	1	+1D
		46	1	Normal female
		47	5	+1C
27.4.65	B.M. Direct	45	1	-1A
		44	1	-2E -1F +1
		43	1	-3C
		47	1	+1C
26.2.65	P.B. Micro + PHA	46	7	Normal female
		46	1	+ fragments
		46	1

FIG. 4. An electron micrograph of mature neutrophil. The segmented nucleus is seen as two separate nuclear areas in the thin section. The nucleus shows the two abnormalities commonly found; the first (A) is a nuclear protrusion filled with nuclear chromatin; the other (B) is a cytoplasmic area surrounded by a double thickness of nuclear membrane. The cytoplasm contains mitochondria, numerous neutrophil granules, and a centriole. $\times 16,000$.

some is a sex chromosome or an autosome because labelling experiments could not be done on the bone marrow. Phenotypically the patient is a normal female. She had never been married. There was no evidence of the presence of double Barr bodies in buccal smears. The nuclear morphology of the polymorphs, although atypical, does not conform to the usual findings in the XXX syndrome. It is to be noted that the patient possibly had a minor population of normal cells in the bone marrow. This could indicate constitutional mosaicism or possibly that the abnormality did not affect precursors of all classes.

The results of a culture of peripheral blood lymphocytes are also given in Table II. Most of the cells were normal female ones except for the presence of a single cell with 47 chromosomes with the same analysis as those in the bone marrow. We do not interpret this double

population as evidence of lymphocyte mosaicism, but concluded that it was derived from primitive cells that had escaped from the bone marrow.

Ten karyotyped cells from a skin culture were all normal; no cells with 47 chromosomes were observed.

The evidence therefore favours the belief that the abnormal cells in the bone marrow do not represent a constitutional anomaly but that they were related to the disease process.

DISCUSSION

There seems little object in trying to assign this case to an exact diagnostic category, *e.g.*, atypical leukaemia or aplastic anaemia. Marrow dysplasia seems an adequate description and since both gold

FIG. 5. A higher power electron micrograph of a nuclear protrusion. In the cytoplasm there is also one of the small osmophilic lamellar bodies (L). $\times 48,000$.

and irradiation may cause both leukaemia and aplasia, either may have had a causative role. The dose in each case is not large but the time intervals (one to five years) are suggestive.

To our knowledge such a high incidence of drumsticks has not been recorded previously. Patients with an XXX chromosomal complement rarely have cells with double drumsticks and on average the incidence of single drumsticks and the mean lobe count is lower than in normal females. This is associated with an increased incidence of sessile nodules. It has been suggested that although the presence of an extra X chromosome inhibits nuclear segmentation, thus preventing the formation of more drumsticks, it may, nevertheless, be expressed in higher incidence of sessile nodules (Mittwoch, 1963).

There is, in fact, no conclusive evidence that the extra chromosome in this case is an X chromosome rather than another member of the C group of chromosomes. In a case of myeloid metaplasia with possible leukaemia, Sandberg, Ishihara, and Crosswhite (1964) have described the presence of an extra C group chromosome in the bone marrow cells, designated C 9. This patient had been treated by radiation to the nose for a basal cell carcinoma. This

was the only cytogenetically abnormal case in a group of 20 patients with myeloproliferative disorders other than leukaemia. The neutrophils showed toxic granulation but no comment was made about the nuclear morphology. Two untreated patients with polycythaemia rubra vera have been found to have a major population of cells in the bone marrow with C trisomy. In neither case were the polymorphs abnormal (Kay, Lawler, and Millard, 1966).

Amongst a series of 75 patients with acute leukaemia Sandberg, Ishihara, Kikuchi, and Crosswhite (1964) have drawn attention to the relative frequency of trisomy C 9 in patients with acute lymphoblastic leukaemia. They suggest that the frequent involvement of C 9 and G group chromosomes in some changes in leukaemia may indicate that the genes carried by these chromosomes are concerned in leucopoiesis.

In the syndrome of trisomy D multiple nuclear projections in the neutrophil leucocytes have been described (Huehns, Lutzner, and Hecht, 1964). A small number of projections are seen in the blood of normal children (Fine, Woo Wang, and Heath, 1966) and their frequency may be increased rarely in persons without chromosome abnormalities (Powar,

FIG. 6. Karyotype of a typical cell from the bone marrow with 47 chromosomes.

1964). The projections differ morphologically from the drumsticks of the superfluous X chromosome and are associated with a characteristic electron-microscopic appearance. What is seen is a round stippled body adjacent to the main nuclear mass and partially enclosed by thin projections from the nucleus. These projections bear a suggestive resemblance to the walls of the nuclear vesicles in the case here described although the contained material is entirely different. We conclude tentatively that an excess of certain components of the genome may influence the formation of nuclear membrane with resultant effects on nuclear structure as a whole.

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