Virchows Arch. A Path. Anat. and Histol. 384, 357–362 (1979)

Virchows Archiv A

Large Basophilic Cells in the Bone Marrow in Iron Deficiency Anemia

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Summary. Many large cells with a strongly basophilic cytoplasm and large nuclei with a delicate chromatin pattern and large nucleoli were studied in 1 μ sections of core biopsies in 8 patients with iron deficiency anemia and in 5 patients with pernicious anemia. In 2 normal controls these cells were rare. Possible features of granulocytic differentiation were assessed with the Giemsa stain and with the naphtol-ASD-chloroacetate esterase reaction which is indicative of early granule formation: the large cells were constantly negative.

In thin sections the morphological appearances of the large basophilic cells are rather similar in such different haematological disorders as pernicious anemia or iron deficiency anemia. A clear difference can be demonstrated by karyometry, which shows that the large basophilic cells in pernicious anemia are significantly larger than those in iron deficiency anemia or in the normal control.

Key words: Large basophilic cells – Pernicious anemia – Iron deficiency anemia – Karyometry.

Introduction

During the microscopical examination of sections of bone marrow cores from the posterior iliac crest we occasionally observed in haematological disorders many groups of large basophilic cells. The increased number of these cells was always coupled to a concomitant increase in haematopoietic tissue and a corresponding reduction of the fatty tissue.

When clinical, haematological and biochemical data were compared it turned out that some of these patients had pernicious anemia (P.A.) but that others

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0340-1227/79/0384/0357/$01.20

had severe iron deficiency (I.D.). Since the changes in the peripheral blood and in the marrow in P.A. are classically described as macrocytic and in I.D. as microcytic the presence of numerous large basophilic cells in the latter group was rather unexpected. The present study is an attempt to clarify this finding, which may present a diagnostic problem to the pathologist looking at the bone marrow as a tissue. The haematologists, examining smears, are not confronted with the tissular aspects of disturbed haematopoiesis in solid tissue.

Materials and Methods

Posterior iliac crest biopsies from 5 patients with pernicious anemia, 8 patients with iron deficiency anemia and 2 normal controls were studied. The pernicious anemia patients all had very low levels of Vit. B 12 and serum folate. The iron deficiency patients all had very low serum iron values, a high IBC, and a history consistent with prolonged bleeding.

The biopsies were done with a Vim Silverman or Yamshidi needle, fixed in mercury chloride formol (B 5) fixative, decalcified in formol EDTA and embedded in paraplast. Sections 5 μ thick were stained with H and E. Tissue blocks showing representative features were reprocessed in glycolmethacrylate (JB_4 Polysciences) and the sections were cut at 1 or 2 μ with glass knives on a Sorvall Porter Blum JB_4 A microtome and stained with H and E, hematoxylin alone, Giemsa and for naphtol-ASD-chloroacetate esterase activity. (Leder, 1964).

The cellularity of haematopoietic tissue versus fatty tissue was expressed on a +/++++ scale as an average of all the marrow cavities of the biopsy core. Haematopoietic tissue occupying 25% of the marrow cavity surface was given a score of +, 50% of ++, 75% of +++ and more than 75% of ++++.

The number, size and composition of the erythrons[1] were assessed especially with respect to the "large basophilic" cells. The morphological characteristics of these cells were studied. For the chloroacetate esterase test special attention was paid as to whether any large cell present in an erythron showed positivity or whether positive large cells were found outside erythrons.

Karyometry was done on Giemsa stained sections, photographed with a 100× immersion objective. The microphotographs were projected on white paper at a final magnification of 8,380. All the rounded non pyknotic nuclei of different marrow cavities were drawn and numbered: in total 218 of a PA-case, 220 of a ID-case and 158 of a normal. Each nucleus was measured by the combined curvimetric-planimetric method of Lange and Lange (1976, 1977) and the volume expressed in mu3 according to the formula $V=(4/9\pi)\cdot(U+\sqrt{\pi\cdot F})\cdot F$, where U is the circumference of the nuclear area and F the surface thereof. The values were submitted to statistical analysis.

Results

The score for haematopoietic tissue was +++ or ++++ in all cases of PA and ID. This was in striking contrast with the normal were it was + or ++ (Figs. 1–3).

In PA the erythrons were numerous and large and were mainly responsible for the total increase in haematopoietic tissue. The contours of the erythrons were irregular and blurred. Adjacent erythrons were often anastomosing or confluent. They were essentially composed of large basophilic cells lying side by side. The cytoplasmic basophilia of these cells was characteristic and of

[1] The term erythron means a cluster composed of normoblasts of different degrees of maturation and is considered to be the tissue unit of erythropoiesis

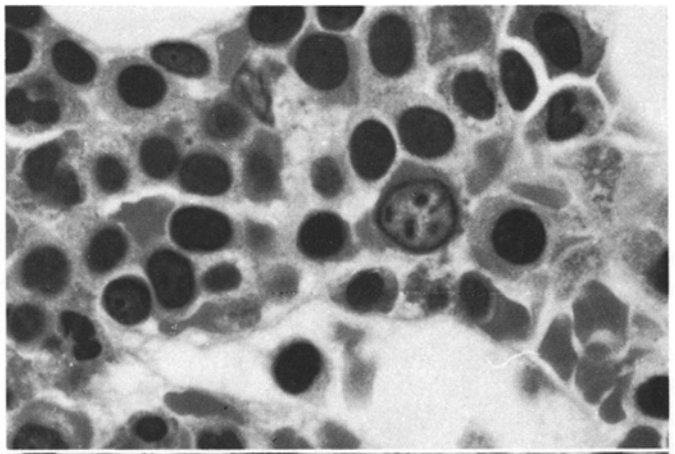

Fig. 1. One large basophilic cell amidst normoblasts in a normal control. H and E ×1418

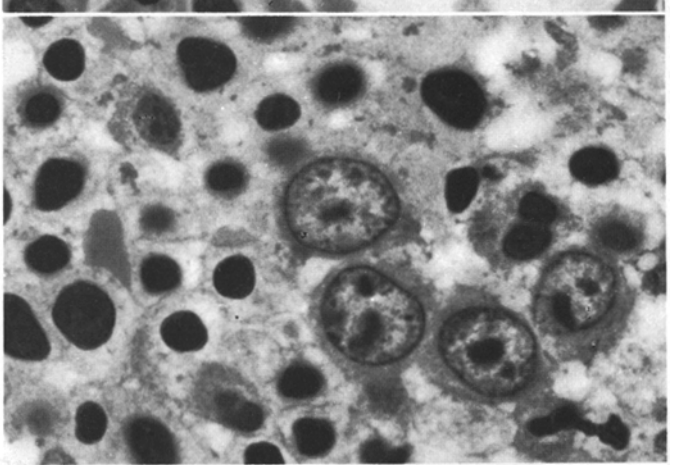

Fig. 2. Group of large basophilic cells mixed with normoblasts in iron deficiency anemia. H and E ×1418

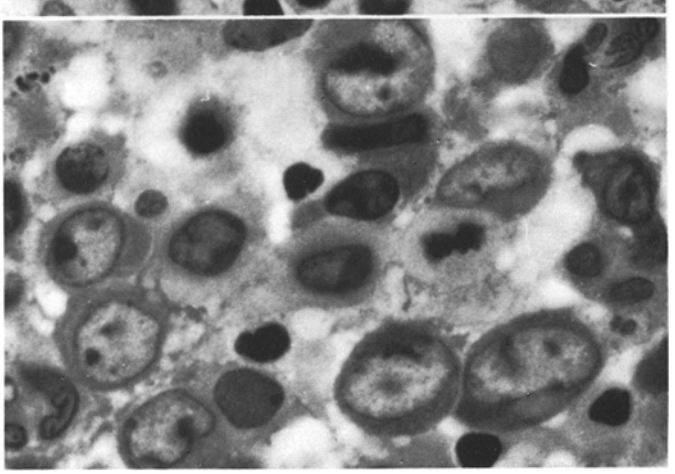

Fig. 3. Group of large basophilic cells with very few normoblasts in *pernicious* anemia. H and E ×1418

the same intensity as in the mature plasma cell. However the delicate chromatin pattern of the nucleus was totally different from the condensed chromatin blocks in the plasma cell. If stained with haematoxylin alone the chromatin pattern of the nuclei of the large cells could be appreciated very well: there was a very narrow regular rim of chromatin against the nuclear membrane which occasionally showed a shallow sharply demarcated notch or even a cleft. Throughout the nucleus the chromatin was finely and regularly dispersed on a clear background. All of the nuclei contained one or more large clearly defined chromatin condensations: they were frequently observed along the border or along the notch or the cleft. Their number and size were very variable from cell to cell, but this was probably due to the thinness of the sections. These condensations probably represent nucleoli surrounded by associated chromatin. Many large cells showed a large halo in the paranuclear zone which stained faintly with eosin. Red cells with advanced differentiation were not only few but had lost their classical nest pattern and were dispersed among the granulocytic series. Among these enlarged metamyelocytes were present. Mitoses were found in the erythrons as well as in the granulocytic series.

In pronounced ID the score of haematopoietic tissue was of the same magnitude as in PA and here again the numerous enlarged erythrons formed the bulk of this tissue. However they retained more the normal compact pattern with discernable borders. Confluence and anastomoses were rare but they also showed the presence of many large cells. These had rounded nuclei with a fine rim of condensed chromatin along the nuclear membrane. Many pinpoint spots of chromatin merged with this rim. On a clear background the chromatin was finely and evenly distributed. Many coarse chromatin condensations probably corresponding to nucleoli were present. The basophilia of the cytoplasm was conspicuous. Apart from the large cells the erythrons contained many more mature nucleated red cells. However sometimes isolated groups only composed of large cells displaying a similarity to leukemic infiltrates were observed.

In the normal bone marrow the score for haematopoietic tissue was + or + +. The erythrons were small and compact and consisted mainly of smaller cells with a homogeneous nucleus containing heavily condensed chromatin and absence of cytoplasmic basophilia. Only occasionally a large basophilic cell with a round or notched nucleus, with finely dispersed chromatin and large nucleoli was found. Exceptionally two or three of these cells were located in a single erythron.

None of the large basophilic cells showed granules. The naphtol-ASD-chloroacetate test was very helpful: it was negative in all these cells grouped in an erythron. A special search was made to find large cells with the characteristic coarse red granules of this reaction outside the erythrons. Some of these cells were indeed found but when the chromatin pattern of these was compared with that of the large basophilic cells in the erythron it became clear that the chromatin of the positive cells was coarser and showed small local clumps. The naphtol-ASD-chloroacetate esterase activity was particularly strong in promyelocytes and myelocytes. The activity in mature neutrophilic granulocytes was generally comparable to that of the younger forms but occasional cells were negative.

Karyometry of the 218 nuclei of a PA case gave values ranging from 15 mu3 to 485 mu3 with an irregular scattering not allowing any grouping. The 220 nuclei of an ID case ranged from 15 mu3 to 360 mu3, and here also a subdivision could not be made. The volume determinations of the 158 nuclei in the normal ranged from 15 mu3 to 265 mu3. The number of the large cells in the PA was 52 (23% of the total) with a mean volume of 307.0 mu3 (sd 121.0 mu3), in the ID the number was 26 (12% of the total) with a mean volume of 229.0 mu3 (sd 70.5 mu3). In the normal only 4 cells (2.5% of the total) could be assigned to this group with a mean volume of 221.0 mu3. The difference in mean volume between the PA-case and the ID-case was statistically significant, $P < 0.001$.

Discussion

Thin sections of bone marrow cores allowed us to study the abnormal findings in severe iron deficiency and pernicious anemia. The thinness of the sections is of major importance in assessing precise cell details. Plastic embedding offers major advantages for thin cutting and is especially suitable for bone marrow (Block, 1976; Burkhardt, 1970).

We were impressed by the fact that the picture of the bone marrow in PA and in ID may look very similar and that without clinical data even an experienced pathologist may be unable to distinguish the two conditions. The cell proliferation can be of the same magnitude in both disorders and can be so intensive in some cases as to mimic leukemia: examples of patients treated for leukemia during pregnancy whereas they were suffering only from a megaloblastic anemia have been reported by Levine and Hanstra (1969).

The most reliable criterion in our hands to distinguish between PA and ID are the composition and structure of the erythrons and not the morphologic characteristics of the large cells.

We consider the large cells to belong to the red series on the following grounds:

1) the cells occur most frequently and most abundantly in situations characterized by an increased and disturbed red cell formation, an argument also used by Lennert (1952).

2) they occur in nests, which is the known growth pattern of the red series. Wienbeck (1938) points out that he is unable to distinguish very young white cells from red and that the only criterion he relies on to recognize early red cell formation is grouping in nests.

3) none of the cells showed granules with the Giemsa stain and all were negative with the naphtol-ASD-chloroacetate esterase stain.

The presence of an increased number of large basophilic cells, sometimes referred to as megaloblastoid cells, in ID has been recognized and documented in man (Chanarin et al., 1965; Van Voolen et al., 1971; Herbert et al., 1971; Hill et al., 1972) and in the rat (Vitale et al., 1966). Herbert et al. (1971) propose that the mechanism is linked to an impairment of the function of the enzyme ribonucleoside reductase which is iron dependent. In a recent paper (1978)

this author presents evidence that in ID there is a folate deficiency, which may be unmasked by the administration of iron.

Our karyometry studies show a statistically significant difference in the volume of the nucleus of the large basophilic cells in PA and in ID and the few cells of this type in the normal have the same volume as in ID.

In conclusion, only a very small number of large basophilic cells are present in sections of normal marrows, but relatively large numbers are found in PA and ID. Those in PA clearly display the increase in nuclear volume which is the characteristic of the disease.

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Received May 31, 1979