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Ultrastructural findings of congenital dyserythropoietic sickle cell beta thal-associated anemia

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Summary. The ultrastructural findings of erythroblasts and reticulocytes in one case of congenital dyserythropoiethic anemia (CDA) associated with a haemoglobinopathy, sickle cell beta thalassemia minor (Type V CDA), is described.

The observations can be summarized as follows:

1) A lot of large breaks are present in the erythroblast nuclear envelope.

2) Nuclear membrane evaginations are filled with dense loose chromatin.

3) Electron-transparent areas (moth eaten chromatin) are evident in dense chromatin.

4) Electron-dense granular material, related to altered haemoglobin chain storage, is evident in the nucleus and in the cytoplasm.

5) Iron deposits are present in mitochondrial matrix.

6) Myelinic figures are present in reticulocyte cytoplasm.

For the first time the ultrastructural findings in this type of associated CDA are described and related to the double origin of clinical symptoms.

Key words: Associated CDA, Erythroblasts, Ultrastructure

Introduction

Since some years the term «Dyserythropoietic anemias (DA)» has been reserved for congenital disorders with peculiar morphological alterations in the nucleus and cytoplasm of erythroblasts, reticulocytes and erythrocytes as primary findings (Lewis et al., 1972; Morgenstern et al., 1973; Kerkoven et al., 1974; Genova et al., 1978; Dell'Orbo et al., 1983).

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On the other hand, a small number of major haemoglobinopathies show alterations of cytoplasmic and nuclear compartments of the erythroblasts and are considered congenital and secondary to altered haemoglobin synthesis, turnover and storage (Fessas, 1963; Polliak and Rachmilewitz, 1973).

The case investigated may be classified as associated congenital dyserythropoiethic anemia (associated CDA) or type V CDA, because of clinical symptoms and the co-existence of haemoglobinopathy and dyserythropoiesis. Descriptions of type V CDA ultrastructural features have increased from 1972 despite the rarity and heterogeneity of these associations (Weatherall et al., 1973; Frish et al., 1974; Polliak et al., 1974; Beuzard et al., 1978).

The purpose of this paper is to describe in detail the ultrastructural findings observed in erythroid cells of a girl affected by a CDA associated with sickle cell beta thalassemia minor, which has not previously described.

Materials and methods

Case report

G.I. was a four-year-old girl, who came to our attention, some weeks after an acute episode of viral hepatitis. The family anamnesis is unknown because she was adopted. The girl showed a diffuse articular joint pain, not accompanied by clinical symptoms of inflammation, with a slight increase in body temperature. Hepatosplenomegaly was also present.

Laboratory data were as follows: Red blood cells: 2.1 x 10^6/mm^3; Reticulocytes: 30%; Haemoglobin: 5g/dl; Htc 20%; White blood cells: 10 x 10^5/mm^3; Differential: 54 N, 36 L, 10 M; Normoblasts: 80,000/mm^3; Conjugated bilirubin: 1.75 mg/100 ml; Direct and indirect Coomb' tests: negative; TIBC: normal range; Osmotic resistances were increased. Haemoglobin electrophoresis: A1 10.5%, A2 23.5%,

F 1%, S 75.5%; Sickle test +++.

The diagnosis on the basis of laboratory investigations and clinical data was: «Drepanocytosis associated with thalassemia minor (thalassodrepanocytosis)».

Sternal marrow examination revealed: «Heinz bodies in erythroblasts and reticulocytes, many clusters of normoblasts with pyknotic nuclei, intracytoplasmic chromatin bridge». Ham test: negative. Iron turnover increased about 9 times.

These data led to the doubt that some form of dyserythropoiesis would be associated to haemoglobinopathies.

The ultrastructural study was performed on bone marrow specimens and peripheral blood in order to answer this clinical question.

Technical methods

Marrow blood was drawn by sternal puncture and anticoagulated with 3.8% sodium citrate (1/10 v/w). Marrow fragments were separated from fat and single cells by a brief centrifugation at low speed and then fixed at 4° C in 2% glutaraldehyde diluted in 0.1 M phosphate-buffered saline at pH 7.4 or in paraformaldehyde-glutaraldehyde 0.1 M cacodylate buffered at pH 7.2 according to Karnovsky (1965).

Peripheral blood was collected by venopuncture and anticoagulated as before described, centrifugated and washed twice in 0.1 M phosphate-buffer saline at pH 7.4. The cells collected in pellet were fixed as above described. The specimens were washed in 0.1 M phosphate-buffered saline at pH 7.4 and postfixed in 1% OsO_4 diluted in 0.1 M phophate buffer saline at pH 7.4.

The pellets were then dehydrated in ethanol, briefly treated with propylenoxide and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate according to Reynolds (1963) and examined using a Philips 300 electron microscope.

Results

While previous sternal puncture was very rich in bone marrow fragments, the second (for electron microscopic investigations) was very poor in bone marrow, yet sufficient for examination. The semithin section light microscopic observations confirmed the Heinz bodies in erythroblasts and reticulocyte cytoplasm. A small number of erythroblasts and erythrocytes was positive to Perls reaction. Some nuclei showed membrane evaginations and nuclear bridges. The erythrocytes had an abnormal shape, as is common in sickle cell anaemias.

Ultrastructural findings

At ultrastructural level peculiar findings both in the nuclear and cytoplasmic compartments were evident.

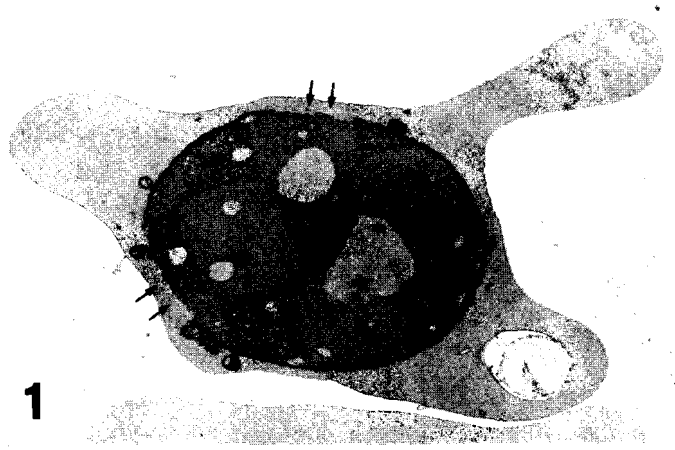

Fig. 1. Erythroblast. Moth eaten areas are evident. (asterisks). Large breaks in perinuclear envelope are also present. (arrows). × 5,500

Fig. 2. Erythroblast. In the loose chromatin granular heterogeneous electron-dense material is evident. (arrows). Mitochondrial matrix presents iron deposits (arrowheads). × 11,500

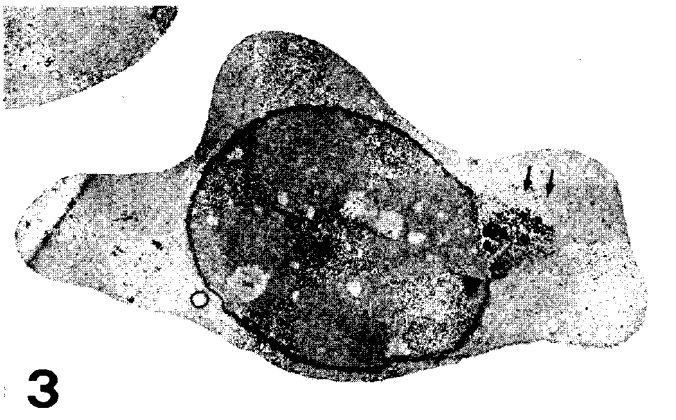

Fig. 3. Erythroblast. Outside the nuclear envelope breaks (arrowhead) granular electron-dense material is evident (arrows). Granular electron-dense material is not limited by any membrane and is in direct contact with dense chromatin. × 5,000

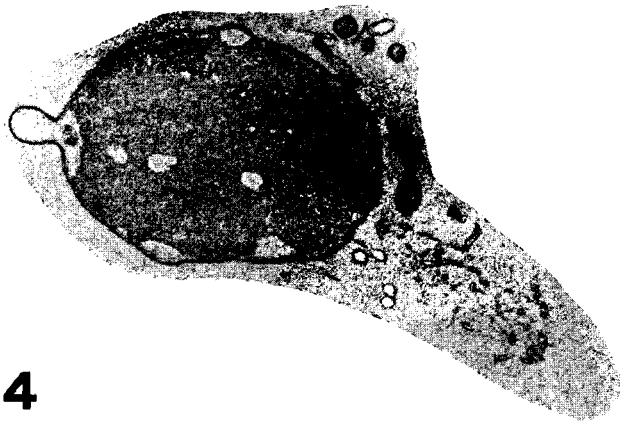

Fig. 4. Erythroblast. A large evagination of nuclear membrane full of loose chromatin is evident. × 5,000

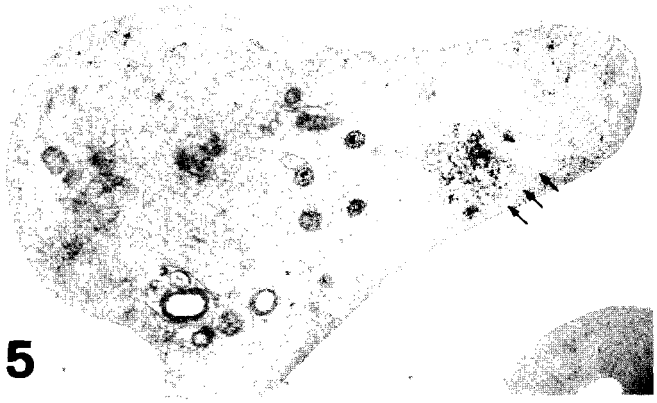

Fig. 5. Reticulocyte. A discrete amount of smooth endoplasmic reticulum and myelinic figures are present in the cytoplasm. Granular, electron-dense material is still evident in the cytoplasm (arrows). × 8,000

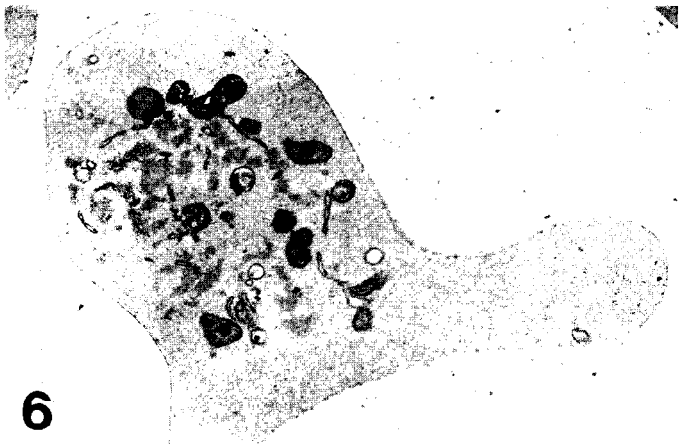

Fig. 6. Reticulocyte. Mitochondrial matrix is full of iron deposits and smooth reticulum cisternae are still evident. × 6,000

Erythroblast nuclei presented dense chromatin marginated near the nuclear envelope, while electrontransparent areas were evident in the central part of the nucleus. The peripheral dense chromatin showed roundish electrontransparent zones varying from 600Å to 8000Å in diameter, randomly distributed (moth eaten areas) (Fig. 1). In the euchromatic areas small granular heterogeneous particles were also evident (Fig. 2). Large breaks in the nuclear envelope were frequently observed.

The breaks faced the cytoplasm which sometimes presented, in correspondence to nuclear membrane breaks, aggregates of granular electron-dense particles (similar to those described in the nuclear area) (Fig. 3). This material was not limited by membrane neither in the nucleus nor in the cytoplasm.

The nuclear membrane showed large evaginations (up to 200 nm), containing chromatin (dense and loose), protruding into the cytoplasm (Fig. 4). The perinuclear cisternae were composed of multiple membrane layers. Sometimes in mitochondria strongly electron-dense iron storages could be observed. Smooth endoplasmic reticulum cisternae, closely packed to form myelinic figures, were present in erythroblast cytoplasm (Fig. 4).

Smooth endoplasmic reticulum, myelinic figures, electron-dense vesicles and sometimes granular heterogeneous particles could be observed in the reticulocytes (Fig. 5). Many iron deposits were present in the mitochondria (Fig. 6).

Discussion

A lot of clinical and laboratory data were indicative of a thalassemia minor associated to drepanocytosis, but abnormal erythrocateresis (evidenced by abnormal hyperbilirubinemia and 9 times increased iron turnover) did not completely corroborate clinical and laboratory diagnosis. Moreover, the great number of circulating reticulocytes (30%) evidences an increased ineffective erythropoiesis, more and more serious than that observed in major haemoglobinopathies. The ultrastructural investigation of erythropoiesis let us observe a lot of morphological alterations in the whole erythroid family.

Some of these alterations are evidently connected to the altered haemoglobin synthesis: the aggregates of electron-dense granular material not limited by membranes, present in nuclear loose chromatin and in cytoplasm, are very similar to the pictures firstly described by Weatherall et al. (1973) and then by Polliak et al. (1974) and Beuzard et al. (1978). They demonstrated that such findings can be related to light haemoglobin chain accumulation either in nuclear or in cytoplasmic areas. In our case also they may be related to free chains of haemoglobin and to the Heinz bodies observed with light microscopy.

The iron storage in mitochondria is also typical of haemoglobinopathies and recurrent in many

haemogblobinopathies (Fessas, 1963; Polliak et al., 1974).

Some other alterations, however, can be related to altered erythopiesis: the moth eaten chromatin was described in CDA I (Lewis et al., 1972; Dell'Orbo et al., 1983) and in CDA II (Berendson et al., 1976) and referred to as peculiar to CDAs and indicative of an error of spiralization and organization of quaternary structure of DNA. The nuclear membrane alterations (interruptions and multiple layer membranes) are frequent in the majority of pure CDA and may result from an adaptive modulation of the cell to altered membrane synthesis (Morgenstern et al., 1973; Fukuda et al., 1984). The evagination of nuclear membrane filled with dense and loose chromatin were never described neither in pure nor associated CDA.

The myelinic figures and the interruptions of nuclear cisternae were also referred to as biochemical defects of membranes which characterize dyserythopoietic anemias (Fukuda et al., 1984).

All these nuclear and cytoplasmic evidences are completely absent in pure haemoglobinopathies.

The present description may be a contribution to the CDA-associated anemia morphology knowledge.

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