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Dihydrofolate reductase activity in the erythroblasts of patients with 5q- syndrome

We demonstrated a qualitative and quantitative cytochemical decrease of dihydrofolate reductase activity in the erythroblasts of 4 patients with the 5q- syndrome compared with 10 normal controls and 8 patients with myelodysplastic diseases. We hypothesized that this enzyme abnormality could be important for understanding the pathogenesis of the syndrome.

Sir,

We carried out an extensive cytochemical study of dihydrofolate reductase (DHFR) in the bone marrow erythroblasts of normal controls, of patients with myelodysplastic syndromes (MDSs) and with malignant transformation of the cells.[1,2] The 5q- syndrome is a particular type of myelodysplastic syndrome characterized by interstitial deletion of the long arm of chromosome 5.[3] A striking number of genes encoding hematopoietic growth factors (IL-3, IL-4, IL-5, IL-9, CSF-2) and growth factor receptors[4] and also the functional human dihydrofolate reductase gene (region q11-q13)[5,6] have been mapped on the long arm of chromosome 5.

The aim of the present work was to study DHFR activity[7] in bone marrow erythroblasts of patients with the 5q- syndrome[8] to see whether there are differences in intensity of DHFR in comparison with the activity in erythroblasts of normal controls and patients with other types of MDS. We carried out the cytochemical reaction on bone marrow imprints of 10 normal controls (male/female= 3/7; median age = 44; range 40-50 years), 8 patients with MDS classified as having refractory anemia (RA) (male/female = 5/3; median age 48; range 30-58 years) and 4 patients with the 5q- syndrome at the onset, not previously treated (all with RA) (male/female = 2/2; median age = 67; range 61-72 years). Two cases had del (5) (q13q33); one case del (5) (q13q31) while the breakpoint was not identified in the other.

Employing a Vickers M86 scanning and integrating microdensitometer at λ= 585±5 nm, the optical density (OD) of 100 erythroblasts for each normal control and patient was counted. The results were expressed as arithmetic means with standard deviation (means±SD). With this cytochemical method, a very weak perinuclear pattern of positivity was observed in the cytoplasm of the 5q- erythroblasts (Figure 1A) whereas the intensity of the reaction was stronger in normal and RA erythroblasts (Figure 1B). In pathologic erythroblasts of RA patients the optical density was significantly higher (OD=97.3±4.4) than in normal erythroblasts (75.9±3).

The optical density of the 4 cases of refractory anemia with the 5q- showed a significant decrease in enzyme activity (45.7±1.7) in comparison with activity in both normal and RA erythroblasts (Figure 2). These differences of enzyme intensity were independent of the maturation stages of the erythroblasts.

The hypolobulate megakaryocytes observed in the bone marrow imprints of the 5q- syndrome also showed a moderately reduced DHFR level compared to the equivalent normal cells (data not shown). The cause of this enzyme reduction is not, at present, known. Further studies are needed to determine the possible association between gene deletion and enzyme decrease; nevertheless other molecular events

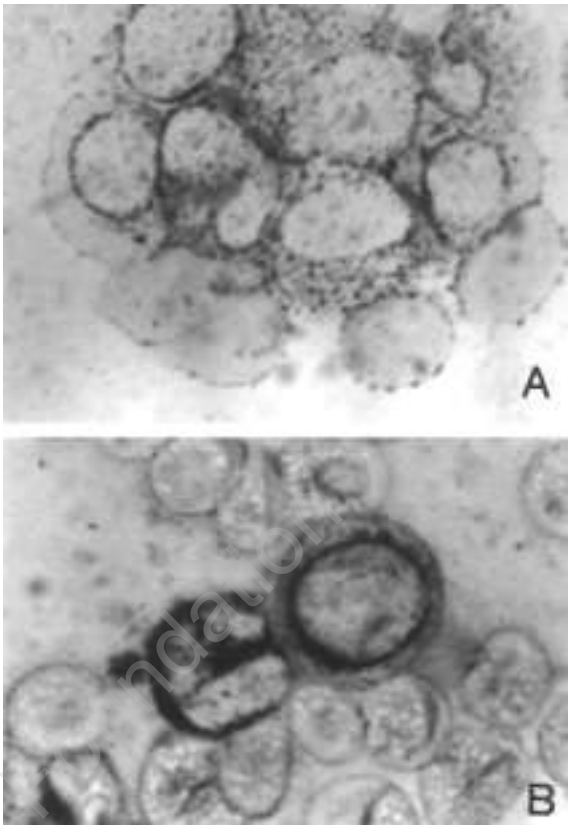

Figure 1. Dihydrofolate reductase reaction. Imprints of bone marrow. A: a very weak perinuclear pattern of positivity in 5q– erythroblasts. Formazan granules of the reaction product are detected around the nucleus (×1,200). B: a very strong perinuclear pattern of positivity in the RA erythroblasts (×1,200).

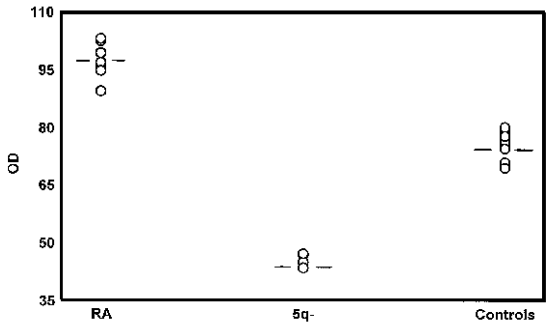

Figure 2. Cytophotometric results of the optical density of DHFR (arbitrary units: means ± SD).

such as instability of the messangers or alterations of the cell cycle could be taken in account. To conclude, for the first time, a reduced expression of DHFR has been demonstrated in 5q- syndrome erythroblasts by qualitative and quantitative studies. This enzyme abnormality could have an important role in the pathogenesis of the disease.

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Key words

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Beneficial effect of low dose G-CSF and cyclosporin-A in a case of chronic neutropenia

A woman suffering from symptomatic acquired chronic neutropenia with a clonal T-cell expansion did not respond to prednisone, full dose cyclosporin-A (CSA) or G-CSF alone. A clinically relevant response was obtained by combining very low doses of CSA and weekly G-CSF administration.

Sir,

Acquired severe chronic neutropenia is a rare autoimmune disease, often associated with a clonal T-cell expansion.[1,2] Infectious complications are frequent and can be fatal. At present, there is not a standard therapeutic approach. Only a few drugs have proven to be beneficial, including cyclosporin-A (CSA) and granulocyte colony-stimulating factor (G-CSF).[3-10] A 59-year old woman was diagnosed in 1992 as having severe neutropenia possibly of an immunologic cause. Indeed, a CD3/CD8 and DR positive T-cell population, demonstrated in bone marrow by flow cytometry, proved to be clonal by T-cell receptor β chain gene rearrangement (R-TcR). No large granular lymphocyte excess was detected in peripheral blood. The patient received a course of prednisone 1 mg/kg and subsequently full dose CSA for two years, without any effect. G-CSF was then added and the dose of CSA was reduced; the neutrophils count reached > 3.000/μL and the patient's clinical condition improved. Because of supply shortage, the patient interrupted G-CSF administration for 10 weeks: neutrophils fell to less than 1.500/μL and a dental abscess appeared. An attempt to lower CSA dose to 25 mg/day was carried out in 1999, but was discontinued because neutrophil count fell below 1,000/μL. Treatment regimens and follow-up data are summarized in Table 1. At present the patient is alive and well; treatment consists of CSA 50 mg daily and G-CSF 300 μg s.c. weekly. Plasma monoclonal CSA is ≅ 30 ng/mL, blood pressure is normal, no side effects are present.

The use of CSA and G-CSF alone or in combination for the treatment of chronic neutropenia has been explored in several studies, which are summarized in Table 2. It appears that our original schedule is the lowest for CSA and among the lowest for G-CSF. Our search for the lowest effective doses of CS-A and G-CSF was justified by the need to avoid possible damage related to lifelong immunosuppression and to

Table 1. Treatment regimens and follow-up data of the patient.

	Therapy	*N/μl*	*Complications/ side effects*	*Hosp.*
Dec 91-Feb 93	None/PDN 75 mg/day	≤ 1,000	Sepsis	5
Feb 93-Feb 95	CSA 400 mg/day	≅1,000	Hypertension, diarrhea	8
Feb 95-Apr 95	None	≤ 250	Sepsis	None
Apr 95-May 98	CSA 50 mg/day + G-CSF 300 μg/week	≅ 3,000	None	None
May 98-Jun 98	CSA 50 mg/day	≅ 1,500	Sepsis	None
Jul 98-Mar 99	CSA 50 mg/day + G-CSF 300 μg/week	≅ 3,000	None	None
Mar 99-Jul 99	CSA 25 mg/day + G-CSF 300 μg/week	≅1,000	None	None
From Jul 99	CSA 50 mg/day + G-CSF 300 μg/week	≅ 3,000	None	None

Hosp.: number of infectious episodes requiring hospitalization. N/μL: neutrophils/μL. PDN: prednisone; CSa: cyclosproin A.