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 receptors4 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 activity7 in bone marrow erythroblasts of patients with the 5q- syndrome8 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...
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 study. This enzyme abnormality could have an important role in the pathogenesis of the disease.

Rosanna Nano, Rosangela Invernizzi,* Alessandro Pecci,* Monica Civallo, Giuseppe Gerzeli

Department of Animal Biology, University of Pavia and Center of Study for Histochemistry, Pavia; *Department of Internal Medicine, University of Pavia, Italy

Key words
Dihydrofolate reductase, cytophotometric analysis, 5q-syndrome, myelodysplastic syndrome

Correspondence
Rosanna Nano, M. D., Dipartimento di Biologia Animale dell’Università di Pavia e Centro di Studio per l’istochimica del C.N.R., Pavia, Italy. Phone: international +39-0382-506315 – Fax international +39-0382-506406 – E-mail: nano@unipv.it

References

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.

Haematologica vol. 85(7) July 2000