MACROPHAGE COLONY-STIMULATING FACTOR: SERUM LEVELS AND cDNA STRUCTURE IN MALIGNANT OSTEOPETROSIS

To the Editor:

Since the discovery of a macrophage colony-stimulating factor (M-CSF) genetic defect in the osteopetrotic op/op mouse,1,2 efforts aimed at identifying a similar deficiency in human patients with infantile malignant osteopetrosis have been made by different groups. A first report by Orchard et al.3 indicated that serum M-CSF levels measured by radioimmunoassay were not reduced in 13 severely diseased patients in whom osteopetrosis was diagnosed during the first months of life. We have measured M-CSF serum levels by enzyme-linked immunosorser assay (ELISA)4 in 14 patients with infantile malignant osteopetrosis (1 to 27 months of age) and seven healthy controls. Our findings confirmed that M-CSF serum levels do not significantly differ between patients and controls.

Several biologically active forms of M-CSF have been recently discovered that include species carrying proteoglycan residues5,6 that bind to components of the extracellular matrix.7 These M-CSF species might have different organ distributions and serve different functions. Therefore, a possibility exists that the exploration of circulating M-CSF will provide little information about tissue-fixed molecules, and normal M-CSF serum concentrations would not exclude a deficiency of M-CSF-induced stimulation of the osteoclastic function in osteopetrotic patients. Candidate abnormalities would affect M-CSF processing and tissue distribution. This possibility prompted us to examine the primary structure of M-CSF cDNA in one osteopetrotic patient.

We performed this analysis in a patient who showed repeatedly, but inconsistently, 30% to 50% reduced M-CSF serum concentrations, whereas his cultured fibroblasts secreted little M-CSF biologic activity. Normal amounts of 4-kb M-CSF mRNAs were present in these cells. The 5' extremity of the patient M-CSF cDNA (nucleotide 1 to 472 of the coding sequence5), which encodes the biologically active amino-terminal domain of the protein,9 was synthesized by reverse polymerase chain reaction. Its sequence was identical to the published one.8 Human M-CSF exon 6 encodes the intermediate part of the protein,10 in which a proteolytic cleavage occurs and generates the soluble growth factor.11 Different splice acceptor sites are used in exon 6, giving rise to mature proteins of different sizes.12 Only full-length species possess proteoglycan attachment sites.8 We looked for modification of exon 6 in the patient's cell DNA that could generate abnormal products. Amplification and sequencing of exon 6 (nucleotide 514 to 1497 of the cDNA sequence) showed no difference from the published sequence.8 Putative proteoglycan attachment sites on Serine 277 and 399 were present. Therefore, it is unlikely that the patient's cells synthesize M-CSF species displaying abnormal maturation and/or restricted organ distribution. Retrovirus-mediated gene transfer was used to express exogenous 4-kb and 1.6-kb human M-CSF cDNAs in patient and control fibroblasts. Both cell types secreted equivalent M-CSF levels in culture supernatants, indicating that M-CSF can be efficiently synthesized in patient's cells. As a complement to previously reported observations, our study suggests that infantile osteopetrosis is not associated with reduced M-CSF secretion into the serum, and provides evidence for the synthesis of normal M-CSF mRNAs and protein products in one patient. However, it does not exclude that M-CSF administration can improve bone resorption in osteopetrotic patients, as recently suggested by preliminary observations from a human trial.12

REFERENCES
10. Ladner MB, Martin GA, Noble JA, Nikoloff DM, Tal R, Ka...


Macrophage colony-stimulating factor: serum levels and cDNA structure in malignant osteopetrosis [letter]

N Naffakh, S Le Gall, O Danos, JM Heard, G Cournot, K Motoyoshi and E Vilmer