## Cellular macrophage colony-stimulating factor and its role

Sir,

Macrophage colony-stimulating factor (M-CSF), which exists in at least three forms, is an important cytokine in hematopoietic and non-hematopoietic systems.

We investigated the distribution of cellular M-CSF (cM-CSF) and its receptor (cM-CSFR). Neither protein was expressed in normal blood (but could be induced by PHA), bone marrow or other adult tissues, but was in fetal liver tissue. On the other hand these proteins were expressed at high level in AML, Hodgkin's disease (HD) and hepatoma samples and at a low level in benign hematopoietic diseases (BHD) and other malignant hematopoietic disease (OMHD). Moreover, high expression was also detected in hematopoietic and non-hematopoietic cell lines (Table 1).[1-4] RT-PCR on 6 cell lines, 12 normal donors and 21 patients with primers for M-CSF and M-CSFR showed diversity of amplification products corresponding to the diversity of protein products, as the alternative splicing.[3]

The above results suggest that co-expression of the proteins was tumor and inflammation associated. The lineage distribution was mainly in granulocytic series, but did occur to a lesser degree in monocytic and in erythrocytic series and, even in some cases, in megakaryocytic series. The cellular localization was mainly in cytoplasm and on membrane, but in some cases,[5] in the nucleus (Table 2).

Recently, Strockbine *et al.* reported that the EBV BARF1 gene encoded a novel, soluble M-CSF receptor.[6] We found that infection by live, but not UV-inactivated HHV-6 increased cM-CSF expression.[7] J6-1 is an EBV and HHV-6 latent infected cell line, which may explain the high expression of M-CSF and M-CSFR. As the infection frequency of EBV and HHV-6 in leukemia and some tumor patients is higher than in other populations, the enhancement of cM-CSF expression might influence the progress of these malignancies.

Membrane-bound M-CSF (mM-CSF) and its receptor were co-expressed in many malignancies and cell lines including J6-1.[1-4] They mediated a contact-stimulating effect designated as *auto-juxtacrine*, which was essential for J6-1 cell proliferation.[8,9] The classical direction of signaling is from cytokine to receptor. However, in this model, mM-CSF acted as a counter receptor.[10] Natural M-CSFR, its monoclonal antibody and recombinant human M-CSF soluble receptor (rh-M-CSFsR), which contained three extracellular Ig-like domains of c-FMS and had binding ability with M-CSF, were used. The treatment of J6-1 cells with rh-M-CSFsR or monoclonal antibodies to block the interaction between mM-CSF and

**Table 1. Distribution of cellular M-CSF and M-CSFR.**

| | | M-CSF | | M-CSFR | | Co-expression^ | |
|---|---|---|---|---|---|---|---|
| *Tissue* | *Case* | *Pos.* | *%* | *Pos.* | *%* | *Pos.* | *%* |
| ***Cell line*** | | | | | | | |
| Hematologic | 7 | 7 | 100 | 7 | 100 | 7 | 100 |
| Non-hematologic | 12 | 9 | 75 | 10 | 83 | 9 | 75 |
| ***Blood*** | | | | | | | |
| Normal donor | 32 | 0 | 0 | 0 | 0 | 0 | 0 |
| PB+PHA | 12 | 10 | 83 | 10 | 83 | 10 | 83 |
| BHD | 17 | 2 | 12 | 0 | 0 | 0 | 0 |
| ALL | 17 | 6 | 35 | 3 | 18 | 2 | 12 |
| CML | 26 | 6 | 23 | 1 | 4 | 1 | 4 |
| AML | 45 | 15 | 33 | 16 | 36 | 12 | 27 |
| OMHD | 23 | 5 | 22 | 1 | 4 | 0 | 0 |
| Hepatoma | 15 | 10 | 67 | 9 | 60 | 7 | 46 |
| Metastatic liver cancer | 5 | 3 | 60 | 2 | 40 | 1 | 20 |
| ***Bone Marrow*** | | | | | | | |
| Normal donor | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| BHD | 14 | 2 | 14 | 3 | 21 | 0 | 0 |
| ALL | 11 | 1 | 9 | 2 | 18 | 1 | 9 |
| CML | 47 | 18 | 38 | 14 | 29 | 11 | 23 |
| AML | 46 | 23 | 50 | 23 | 50 | 19 | 41 |
| OMHD | 22 | 2 | 9 | 2 | 9 | 1 | 5 |
| ***Tissue*** | | | | | | | |
| Fetal liver | 5 | 3* | 60 | 1 | 20 | 1 | 20 |
| Hepatoma | 5 | 4° | 80 | 5 | 100 | 4 | 80 |
| NHL | 17 | 3 | 18 | 2 | 12 | 2 | 12 |
| HD | 12 | 9 | 75 | 9 | 75 | 9 | 75 |
| Control tissue# | 3 | 0 | 0 | 0 | 0 | 0 | 0 |

*BHD: benign hematopoietic disease; OMHD: other malignant hematopoietic disease; PB: normal donor's peripheral blood mononuclear cells; PHA: phytohemagglutinin; *fetal liver gave a weak positive reaction: positive frequency rate 12.6±0.9; score 15.3±1.3; °hepatoma tissue appeared strongly reactive: positive rate 53.0±14.3, score 67.1±15.9, two of the positive cases' reaction appeared to have mainly nuclear M-CSF. #Control tissue: one case each of hyperplasia of breast biopsy, phimosis and mucormycosis. ^Co-expression of M-CSF and M-CSFR. Cellular M-CSF and M-CSFR were investigated via Avidin-Biotin-Complex immuno-peroxidase assay with anti-M-CSF McAb (B5) and anti-M-CSFR McAb (RE2).*

**Table 2. Lineage and cellular distribution of M-CSF in bone marrow.**

| *Group* | *Case* | *Pos.* | *Gran.* | *Mono.* | *Ery.* | *Nuc.* | *Cyto.* | *Memb.* |
|---|---|---|---|---|---|---|---|---|
| Don (PB+PHA) | 12 | 10 | 10 | 1 | 0 | 7 | 10 | 5 |
| Hema-cell line | 6 | 6 | - | - | - | 3 | 6 | 5 |
| BHD | 14 | 2 | 2 | 0 | 0 | 0 | 2 | 2 |
| OMHD | 22 | 2 | 2 | 0 | 1 | 0 | 2 | 2 |
| Leukemia | | | | | | | | |
| ALL | 11 | 1 | 1 | 0 | 1 | 0 | 0 | 1 |
| CML | 47 | 18 | 18 | 1 | 4 | 3 | 18 | 17 |
| M0-3 | 34 | 16 | 16 | 1 | 0 | 0 | 14 | 13 |
| M4-5 | 11 | 6 | 6 | 4 | 0 | 1 | 3 | 4 |

*Study by ABC immunoperoxidase assay; 200 cells were investigated.*

M-CSFR on cell surface resulted in the inhibition of cluster forming process. Further work revealed that rh-M-CSFsR decreased the multinuclear cell ratio and HLA-DR expression in J6-1 cells and caused intracellular pH changes. A rapid dose-dependent rise of cytosolic [$Ca^{2+}$] was observed within 90 seconds. Western blot experiments revealed that rh-M-CSFsR caused the tyrosine-phosphorylation of multiple cytoplasmic proteins with molecular weights of approximately 45 and 55-90 kD, suggesting the involvement of PTK in the signaling.

Our results suggest that mM-CSF/M-CSFR should play a role in molecule adhesion. The cell surface is particularly important in these topobiological interactions because it mediates signals and the adhesion molecules link with other surfaces to form tissue. This suggests that mM-CSF/M-CSFR might play a morphoregulatory role in some tumors and influence metastasis.

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*Cellular macrophage colony-stimulating factor, cytokine, tumor, c-fms*

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## Numb chin syndrome in 4 patients with hematologic malignancies

Sir,

Numb chin syndrome (NCS) is a rare cranial neuropathy usually associated with malignant neoplasias.[1-4] It is characterized by unilateral or bilateral sensory loss and paresthesias of the chin restricted to the territory of the mental branch of the mandibular nerve and can appear together with other signs of neoplastic dissemination or constitute the presenting symptom of the disease. We describe 4 patients with lymphoma and multiple myeloma who had this syndrome.

*Patient #1*. A 64-year old man was admitted to our hospital with a relapse of aggressive lymphoma. After a brief improvement obtained with chemotherapy, lymphomatous relapse was evident coinciding with new-onset paresthesia of the right side of the chin and lower lip. Magnetic resonance imaging (MRI) showed an infiltration of the jaw. The disease progressed and the patient died of an opportunistic infection 2 months after the appearance of the neurological signs.

*Patient #2.* A 19-year old male with relapsed Burkitt's lymphoma, diagnosed from a computed tomographic (CT) scan that showed an abdominal mass, developed numbness over the chin. MRI showed mandibular infiltration. Cerebrospinal fluid analyses were normal. There was no response to the treatment and the patient died 5 months later.

*Patient #3*. A 57-year old man, who had received a heart transplant four years earlier was admitted to hospital because of a large cell lymphoma. The patient presented, together with other signs of progression, bilateral numbness of the chin. An MRI showed tumoral infiltration of the mandible. He died two months later of an opportunistic infection (with the lymphoma in progression).

*Patient #4.* A 54-year-old man with early relapsed