Decreased serum soluble macrophage colony-stimulating factor receptor level in leukemia patients

We developed an enzyme-linked immunosorbent assay (ELISA) technique that specifically determines the level of serum soluble macrophage colony-stimulating factor receptor (sM-CSFR). From 102 normal donors, 94 serum samples had detectable sM-CSFR and the average level was 0.47±0.28 ng/mL. Immunoprecipitation and Western-blot assay confirmed that the serum sM-CSFR was a ~90kDa protein. Sera from 75 out of 118 leukemia patients tested negative. The average sM-CSFR levels of AML and ALL patients were 0.17±0.16 ng/mL and 0.22±0.23 ng/mL, respectively: these were significantly lower than that of normal donors (p=0.0001 and p=0.002, respectively).

Many cytokine receptors can be synthesized as soluble forms. Evidence is accumulating that soluble receptors can act in vivo to regulate the biological activity of their ligand and may sometimes be indirect markers of an underlying disease.[1,2] Our previous study has suggested that co-expression of cellular M-CSF and its receptor is tumor associated.[3,4,5] We now determined the levels of serum soluble M-CSFR (sM-CSFR) in normal human and leukemia patients. Using two specific anti-M-CSFR antibodies and rhM-CSFR standard,[6,7] we developed a sandwich ELISA that can quantify the level of sM-CSFR in serum. Our M-CSFR-specific ELISA has high specificity and sensitivity (analytical detection limit was 0.1ng/mL), a broad linear range and good reproducibility. sM-CSFR was identified and measured in 102 normal human serum samples. We assigned a value of 0 ng/mL to any sample below the detection limit. This enabled us to calculate mean±SD serum sM-CSFR concentration. The mean value was 0.47 ± 0.28 ng/mL. There was no significant difference either between male and female groups or between any two age groups. The sera were immunoprecipitated and immunoblotted with anti-M-CSFR antibodies. A single protein band of ~90 kDa reacted with the anti-M-CSFR MAb in each positive sample, but not in the sM-CSFR-negative samples. The relative intensities reflected the amounts of sM-CSFR, supporting the validity of the quantity obtained by the ELISA. Our finding indicates that sM-CSFR circulates in normal human blood.

A total of 143 serum samples from patients with iron deficiency anemia (IDA) and leukemia were screened for the quantities of sM-CSFR via ELISA (Figure 1). The average sM-CSFR level of 25 IDA patients was 0.59±0.40 ng/mL and no significant difference was found compared with normal value (p=0.2). Of 60 AML samples, 41 samples showed negative. The mean level was 0.17±0.16 ng/mL and was significantly lower than the normal value (p=0.0001). Similarly to the cases of AML, sM-CSFR was not detected in 25 ALL samples. The mean value was 0.21±0.22 ng/mL and was significantly lower than that of normal individuals (p=0.002). From 22 CML patients, 9 samples did not have detectable sM-CSFR. The mean level was 0.32±0.30 ng/mL but

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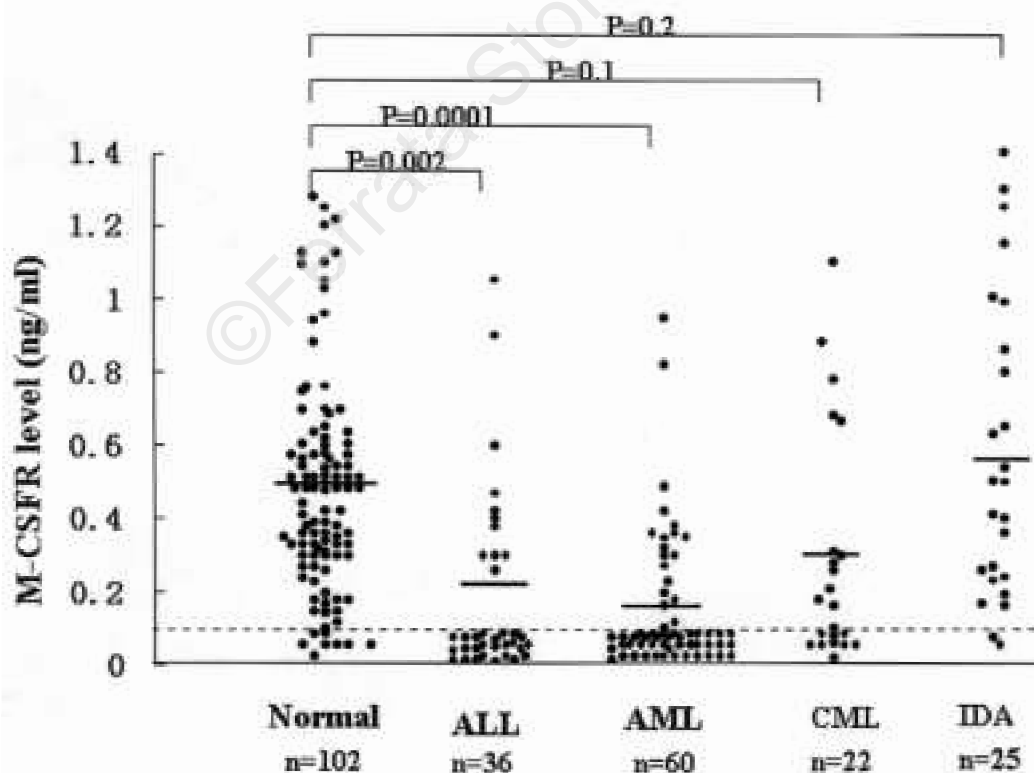

Figure 1. Serum level of soluble M-CSFR in normal donors and leukemia patients. Solid circles indicate individual values. Horizontal bars indicate the groups' mean value. The line of dots indicates the detection limit.

there was no significant difference compared with the normal value (p=0.1).

To determine the correlation of sM-CSFR levels with FAB subtypes, we compared the mean levels in M0-M5 with the normal value. All subtypes had significantly lower values than normal, but no significant difference was found between any two subgroups. Finding that most of the samples with lower levels were distributed in M4 or M5, we further applied Pearson's χ^2-test to compare the ratio of lower sM-CSFR in M4-M5 with other subtypes. The relative normal range was defined as normal average -SD ~ normal average +SD, i.e. 0.19 ng/mL ~ 0.75 ng/mL. Among AML patients, M4 patients had the highest ratio of lower sM-CSFR level (<0.19 ng/mL) (11/12, 92%), followed by M5 patients (8/9, 90%). Decreased sM-CSFR was observed in 20 of 22 M4-M5 cases (91%) but 26 of 38 in other AML subtypes (66%). The ratio of lower sM-CSFR in M4-M5 was significantly higher than that in other AML subtypes (p=0.04). There was an overlap between normal values and those obtained from patients with AML and ALL. We analyzed the information of the overlapping values with normal in acute leukemia. No significant correlation of the ratio of samples with relative normal level to sex, age, WBC counts or CR rate was found. However, the CR rate by initial induction therapy in the group with decreased sM-CSFR level seemed lower than that in the group with a relatively normal sM-CSFR level, but this needs further study since a statistical difference was not obtained (53.1% vs. 76.9%, p=0.33 in AML, 60.0% vs. 80.0%, p=0.42 in ALL).

To our knowledge, this study is the first description of serum sM-CSFR in normal human subjects and in patients with leukemia. Our previous study showed that membrane-bound M-CSFR was expressed at high levels in AML and ALL.[3,4] Another report showed that c-fms was expressed at a maximum level in acute leukemia with features of monocytic differentiation (M4 and M5).[8] The central conclusion from our data is that serum levels of sM-CSFR are significantly decreased in patients with AML and ALL It seems that sM-CSFR may be involved in hematopoiesis. It remains to be determined what role soluble M-CSFR may play in modulating M-CSF function in hematopoiesis and in acute leukemias.

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