short report
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TRILINEAGE RESPONSE TO GRANULOCYTE COLONY-STIMULATING FACTOR ADMINISTRATION IN A PATIENT WITH MYELODYSPLASTIC SYNDROME

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ABSTRACT

We report a 72-year-old man with refractory anemia with excess of blasts who presented severe pancytopenia and pneumonia and received granulocyte colony-stimulating factor (G-CSF) treatment over a 6-week period. In addition to a dramatic increase in mature neutrophils, platelet count and hemoglobin level, the patient achieved a hematological remission which continued for more than 5 months despite discontinuation of the treatment. This observation confirms that in some cases during G-CSF treatment erythropoiesis and thrombopoiesis may improve in addition to the expected effect on neutrophils. While the patient remained in hematological remission, bone marrow examination revealed trilineage dysplasia. This finding suggests that the hematological remission in this patient may not have resulted from a recovery of non-clonal hematopoiesis of a normal clone, but may have derived instead from the monoclonal hematopoiesis of a neoplastic clone.

Key words: granulocyte colony-stimulating factor, myelodysplastic syndrome, RAEB

Recombinant granulocyte colony-stimulating factor (G-CSF) has recently become available as a therapeutic agent for clinical use. G-CSF acts mainly on differentiated granulocyte precursors and has major differentiative as well as proliferative effects on later myeloid precursor cells. Favorable effects on hematopoiesis in patients with myelodysplastic syndrome (MDS) have been demonstrated, including improvement of neutrophil levels and marrow myeloid maturation; there is some evidence that patients with severe infectious problems will benefit from this treatment.[1,2] Significant increases in erythrocytes and platelets were not expected in clinical trials of G-CSF.[1] We report an interesting case of MDS in which a significant increase of erythrocytes and platelets, as well as granulocytes was observed during administration of G-CSF and maintained despite discontinuation of the treatment.

Case report

A 72-year-old man was admitted in November 1993 for evaluation of asthenia, weight loss, fever and cough. Laboratory data on admission were as follows: white blood cell (WBC) count 1.06×10^9/L with 22% neutrophils, 15% of which showed a pseudo-Pelger anomaly, 1% eosinophils, 1% basophils, 65% lymphocytes, 7% monocytes, 4% blasts; red blood cell (RBC) count 2.7×10^{12}/L, hemoglobin 9.5 g/dL, mean corpuscular volume (MCV) 112 fL, reticulocytes 0.9%, platelets 91×10^9/L. Serum vitamin B_{12} level was 328 pg/mL (normal values 271-966) and folate level was 6.5 ng/mL (3-17.5). While waiting for these determinations to be made the patient, who presented macrocytic anemia, received 3 parenteral injections of cyanocobalamin and folic acid, and began maintenance therapy with pyridoxine. An aspirated bone marrow specimen revealed hypocellular marrow with a myeloid to erythroid ratio of 1:3, myeloid hypoplasia with dysgranulopoiesis, binuclear erythroid precursors, bizarre nuclear shapes, cytoplasmic bridging, abnormally dense chromatin with asynchronous cytoplasm.

Megakaryocyte abnormalities with increased

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micromegakaryocytes and large mononuclear cells were present; 18% of blasts were non erythroid blast cells. Cytochemical techniques showed a weak diffuse positivity to the Sudan Black B reaction in 20% of marrow blasts, PAS and non-specific esterase staining negativity; 49% of erythroblasts contained iron granules after Perls' staining, but no ringed sideroblasts were found. Cytogenetic examination revealed a normal marrow karyotype in all metaphases analyzed.

The patient was diagnosed with refractory anemia with excess of blasts (RAEB) according to the FAB classification of MDS.

After admission pancytopenia worsened: neutrophils gradually decreased to 0.1×10^9/L, hemoglobin to 8.6 g/dL and platelets to 20×10^9/L. The patient developed bilateral bronchopneumonia caused, as expectorated sputum culture showed, by *Staphylococcus epidermidis*. Further investigation with bronchoscopic biopsy and brushing documented *Aspergillus* as well. A bleeding gastric ulcer also developed. Antibiotic and antifungal treatment proved ineffective. The condition appeared very serious when subcutaneous administration of G-CSF at 5 μ/kg/day was started. As shown in Figure 1, neutrophil count rose into the normal range within 25 days of treatment; moreover, an unexpected significant response was also observed in the erythroid and megakaryocytic lineages. The platelet count and Hb concentration both increased dramatically shortly after G-CSF administration. Maximum values of PLT and Hb were 250×10^9/L and 13 g/dL, respectively. G-CSF treatment was discontinued after 40 days, with resolution of the pneumonia. The patient's state of health quickly recovered, he no longer needed blood transfusions and no longer had an infection; 3 months later laboratory data confirmed that total leukocyte count, hemoglobin and platelets were maintained in the normal range. Morphological examination of peripheral blood revealed macrocytosis of red cells, some circulating neutrophils showed a pseudo-Pelger anomaly and were degranulated, but circulating blasts were not observed.

Bone marrow aspiration revealed a marked improvement in marrow cellularity, with an increase in mature myeloid precursors and a decrease in blast percentage to 7%; trilineage morphological dysplasia persisted, though reduced. Six months after G-CSF administration the patient showed signs of relapse and progressed to overt leukemia. Evolution to acute myeloid leukemia (AML) is part of the natural history of this disease,[3] especially in those patient with a higher initial bone marrow blast cell percentage.[4] Randomized control trials comparing G-CSF with supportive care will be needed to determine whether such treatment alters this predisposition.[5] MDS patients are heterogeneous, and it is possible that selection factors could contribute to different response rates to G-CSF administration; however, a recent multi-center study has shown that this treatment did not accelerate the development of overt leukemia.[6]

Discussion

We present a case of RAEB in which G-CSF administration resulted in a multilineage response, with maintenance of complete hematological remission for more than 5 months despite discontinuation of the biological agent.

This observation, similar to those reported by Washizuka,[7] Rey[8] and Chiba,[9] confirms that in some cases during G-CSF treatment erythropoiesis and megakaryocytopoiesis may improve in addition to the expected effect on neutrophils. The long-term multilineage response described here is a very rare event.

An increase in granulocytes as well as reticulocytes and/or platelets following G-CSF administration is reported in some patients with MDS, but this response is transient in most cases. Improvements in blood counts were dependent on continued treatment, while cessation of the CSFs brought blood counts rapidly to baseline values.[1]

This trilineage response remains a matter of speculation in the absence of clonal markers in our patient and can be attributed either to a preferential stimulation of the normal residual population, or to maturation of the leukemic clone.[9] The presence of abnormal hematopoiesis during hematological remission, as sug-

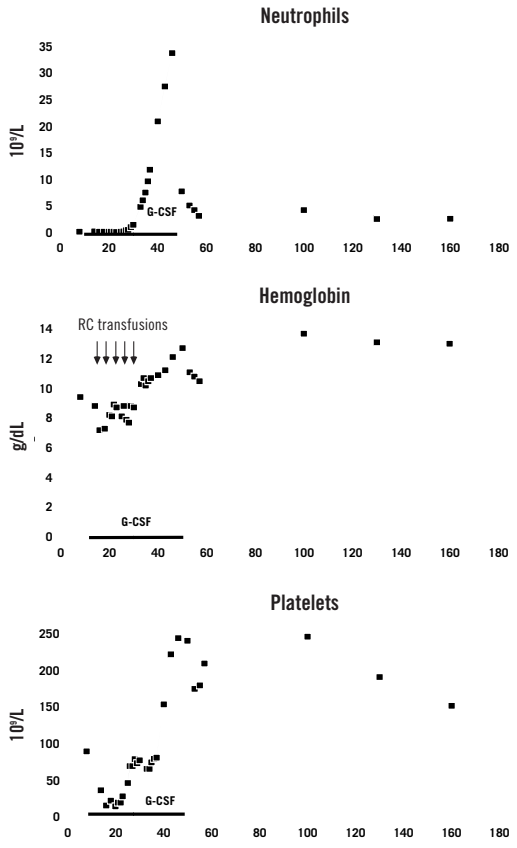

Figure 1. Hematological response during the entire clinical course of the reported patient. The bar indicates subcutaneous administration of G-CSF. The arrows mark red cell transfusions.

gested by persistent trilineage dysplasia and high MCV and MCH values, might indicate that G-CSF stimulated clonal myelodysplastic hematopoiesis, in agreement with what has been described in the literature.[1]

Human marrow culture studies have recently demonstrated that G-CSF is both a late-acting cytokine that regulates proliferation and maturation of neutrophil progenitors, and an early-acting synergistic factor able to trigger the cycling of dormant progenitors.[10] Hogge *et al.*[11] showed that selective elevation of G-CSF in long-term culture markedly expanded both erythroid and granulocytic progenitors. Though the response in this patient was very unusual, it is possible that the abnormal clone in this case expressed G-CSF receptors and that this cytokine stimulated the proliferation of neoplastic hematopoietic stem cells and their subsequent differentiation into erythroid, granulocytic and megakaryocyte precursors.[12]

Moreover, dramatic responses to G-CSF have been observed after severe infections: a similar response to G-CSF after pneumonia was reported by Koike *et al.*[13] These effects may be explained by a concomitant increase in cytokine production during the inflammatory reaction, which may act co-operatively with G-CSF to improve cytopenia. The factors involved in this synergistic activity remain to be identified. The mechanism by which our patient achieved a hematological remission through clonal hematopoiesis is unclear, but results recently presented by Bessho[14] suggest that G-CSF may also exert beneficial effects in the treatment of myeloid leukemias by inducing apoptosis of leukemic cells, with loss of the neoplastic clone's capacity for self renewal.

In conclusion, we observed a patient with RAEB who showed a favorable multilineage hematopoietic response to G-CSF; it is necessary to further identify populations with these characteristics among patients with MDS and perhaps among those with AML as well.

References

1. Negrin RS, Haeber DH, Nagler A, et al. Maintenance treatment of patients with myelodysplastic syndromes using recombinant human granulocyte colony-stimulating factor. Blood 1990; 76:36-43.
2. Harmenberg J, Hoglund M, Hellstrom-Lindberg E. G and GM-CSF in oncology and oncological haematology. Eur J Haematol 1994; 55(Suppl):1-28.
3. Resegotti L. The therapy of hematological malignancies in the elderly. Haematologica 1993; 78:141-4.
4. Vadhan-Raj S, Broxmeyer HE, Spitzer G, et al. Stimulation of nonclonal hematopoiesis and suppression of the neoplastic clone after treatment with recombinant human granulocyte-macrophage colony-stimulating factor in a patient with therapy-related myelodysplastic syndrome. Blood 1989; 74:1491-8.
5. Negrin R, Stein R, Vardiman J, et al. Treatment of the anemia of myelodysplastic syndromes using recombinant human granulocyte colony-stimulating factor in combination with erythropoietin. Blood 1993; 82:737-43.
6. Gabrilove J. The development of granulocyte colony-stimulating factor in its various clinical applications. Bood 1992; 80:1382-5.
7. Washizuka T, Koike T, Toba K, Nagai K, Takahashi M, Shibata A. A rise of erythrocytes and platelets in a patient with myelodysplastic syndrome during the administration of G-CSF. Am J Hematol 1992; 39:153-4.
8. Rey C, Bader-Meunier B, Tertian G, Mielot F, Tchernia G,

Yver A. A trilineage response to recombinant human granulocyte colony-stimulating factor in a patient with myelodysplastic syndrome. Am J Hematol 1993; 43:76-7.
9. Chiba S, Inamori K, Mitani K, Hirai H, Yazaki Y. Marked and reproducible increase in trilineage blood cell counts by administration of granulocyte colony-stimulating factor in a patient with refractory anaemia with excess blasts in transformation. Br J Haematol 1994; 86:665-7.
10. Ogawa M. Differentiation and proliferation of hematopoietic stem cells. Blood 1993; 81:2844-53.
11. Hogge DE, Cashman JD, Humphries RK, Eaves CJ. Differential and synergistic effect of human granulocyte-macrophage colony-stimulating factor and human granulocyte colony-stimulating factor on hematopoiesis in human long-term marrow cultures. Blood 1991; 77:493-9.
12. Bessho M, Itoh Y, Kataumi S, et al. A haematological remission by clonal hematopoiesis after treatment with recombinant human granulocyte macrophage colony stimulating factor and erythropoietin in a patient with therapy related myelodysplastic syndrome. Leuk Res 1992; 16:123-31.
13. Koike T, Kashimura M, Washizuka T, Takahashi M, Shibata M. A rise of erythrocytes and platelets in patient with myelodysplastic syndrome by the administration of G-CSF. In: Miyazaki T, Takaku F, Uchino H, eds. Myelodysplastic syndrome and cytokines. Amsterdam:Elsevier, 1991:289-92.
14. Bessho M, Yoshida S, Sakate K, Murohashi I, Jinnai I, Hirashima K. Suppression of the development of murine myeloid leukemia with granulocyte colony-stimulating factor by inducing apoptosis of leukemic cells. Leukemia 1994; 8: 1185-90.