Levels of Soluble Stem Cell Factor in Serum of Patients With Aplastic Anemia

By Aleksandra Wodnar-Filipowicz, Sue Yancik, Yolanda Moser, Verena dalle Carbonare, Alois Gratwohl, André Tichelli, Bruno Speck, and Catherine Nissen

Aplastic anemia (AA) is a rare bone marrow (BM) disorder characterized by an unexplained failure of hematopoietic precursors to proliferate. In vitro growth of AA BM cells can be improved by the addition of the hematopoietic growth factor SCF (stem cell factor), which suggests that deficiency of SCF may be one of the underlying causes of the disease. In this study, we measured the concentration of SCF in sera of patients with severe AA. One hundred twenty-eight serum samples from 32 patients, at diagnosis and following therapy, were analyzed. Before treatment, SCF levels varied between 0.33 and 6.1 ng/mL; no correlation between hematopoietic function and SCF serum levels was apparent. Therapy with antilymphocyte globulin (ALG) or bone marrow transplantation (BMT) did not result in a recognizable pattern of changes in SCF levels. However, serum concentration of SCF in many patients with AA was at the low range of control serum levels determined in healthy blood donors. Of 128 AA serum samples tested before and after therapy, 107 were below the mean normal value of 3.3 ng/mL, including 26 samples below the minimum normal value of 1.3 ng/mL, as estimated in 267 controls. We also found that SCF levels in peripheral blood serum correlate well with factor concentration in the BM plasma. Clinical observations suggest that higher SCF serum levels are often associated with a better clinical status of the patients in terms of survival and transfusion requirements. The data indicate that a deficient production of soluble SCF may contribute to AA in some patients; thus, suggesting a potential therapeutic benefit of SCF in this disorder.

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The pathogenesis of hematopoietic failure in aplastic anemia (AA) is largely unknown. An unidentified defect in blood cell progenitors that compromises their proliferation is a likely primary cause of the disease. In some, but not all, cases, this defect is accompanied by dysfunction of the microenvironment in the bone marrow (BM). The abnormalities can either include cell- or soluble factor-mediated inhibition of hematopoietic maturation or be associated with a lack of optimal support of hematopoiesis by environmental cells or their factors.

For young patients who have a matched donor, bone marrow transplantation (BMT) is the treatment of choice. BMT offers the only chance of cure. For patients who are not eligible for BMT, the treatment with the two immune-regulatory drugs horse antilymphocyte globulin (ALG) and cyclosporine A (CyA) has substantially improved prognosis; although this alternative treatment leaves the majority of patients with residual disease activity. The in vivo effect of ALG and CyA is not fully understood; however, their mechanism of action may involve both suppression and stimulation of production of hematopoietic growth factors.

This supports the assumption that abnormalities in growth factor levels may play a role in the pathophysiology of AA. Growth factors with hematopoietic activity, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3), and granulocyte colony-stimulating factor (G-CSF), have recently been introduced into clinical trials for the treatment of AA. Although their effect is usually transient, they have proven beneficial for some patients, particularly with regard to survival of severe neutropenia. The therapeutic value of growth factors in improvement of BM function is being further investigated.

Stem cell factor (SCF) is a newly identified growth factor, acting as a ligand for c-kit receptor. It is produced by BM stromal cells and has been implicated in the maintenance of an optimum hematopoietic environment. SCF acts at an earlier differentiation level than the other known growth factors and therefore has the capacity to stimulate the earliest hematopoietic stem cells; that is, those responsible for long-term marrow reconstitution. In synergy with other hematopoietic growth factors in vitro, it promotes growth of normal human BM cells. It also stimulates colony formation by precursor cells from patients with hypoproliferative disorders: Diamond-Blackfan anemia and AA. Based on results of preclinical studies, a therapeutic value of SCF has been postulated.

To characterize the function of aplastic BM stroma in terms of SCF production and to examine the role of SCF in the pathophysiology of AA, we have determined the concentration of soluble factor circulating in the serum of patients with AA. The level of SCF was measured in sera of 32 patients with severe AA before treatment and at several time intervals following ALG therapy (26 patients) or BMT (6 patients). Concentration of SCF in AA peripheral blood serum was compared with values observed in healthy controls, as well as with SCF levels found in the BM plasma of patients with AA. The study represents the first report on endogenous in vivo SCF production in AA and should help in assessing the usefulness of the factor in the treatment of this disease.

Materials and Methods

Patients. Thirty-two patients from a prospective study for the treatment of patients with severe AA, initiated at the Kantonsspital...
tions in Boulder CO, using an enzyme immunoassay, the concentration of SCF in peripheral blood serum. Using an enzyme immunoassay, the concentration of SCF was determined in 128 serum samples from 32 patients with AA. For all patients, samples were collected at presentation and at various time intervals after treatment with ALG (n = 26) or allogeneic BMT (n = 6). The results are presented in

Table 1. Patient Characteristics

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<td>ALG x 2</td>
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Abbreviations: ALG, antilymphocyte globulin; BMT, bone marrow transplantation; R, remission; R^a, remission with pancytopenia; R^b, remission with pancytopenia and transfusion dependence; PNH, paroxysmal nocturnal hemoglobinuria; PNH^a, PNH detected only in laboratory tests; CyA, cyclosporine A; A, androgens; Epo, erythropoietin; UPN, unique patient number.

* Values in parentheses indicate time between therapy and death.

Basel in 1976, were included in the present analysis (Table 1). Before treatment, all patients had severe AA, as defined by the Camitta criteria, and all were transfusion dependent. Thirty-one patients had idiopathic acquired AA, and in one case (patient 18), the disease was drug related. Twenty-six patients were treated with horse antilymphocyte globulin (ALG; Lymphoser Berna, Berne, Switzerland) in combination with high-dose corticosteroids, following a standard protocol. Six patients underwent BMT.

Controls. Peripheral blood from 267 normal individuals were used with informed consent.

Peripheral blood serum and BM plasma. Serum was collected from heparinized peripheral blood after centrifugation for 10 minutes at 2,500 rpm. Plasma was obtained from heparinized BM after centrifugation for 10 minutes at 1,500 rpm. Samples from patients and control donors were aliquoted and stored at −70°C until used. Analysis has been performed immediately after thawing.

Determination of SCF. SCF concentrations in control and aplastic serum and plasma were measured under identical conditions in Boulder CO, using an enzyme immunoassay. Immunol 4 Removawell strips (Dynatech Laboratories, Inc., Chantilly, VA) were coated with affinity-purified polyclonal anti-SCF antibody raised against CHO cell-derived recombinant human SCF (rhSCF). Sample diluent was added to all test wells followed by the addition of samples, standards (known concentration of CHO cell-derived rhSCF added to human serum depleted of SCF), and controls. Microtiter test plates were incubated for 4 hours and then washed. The 7H6 monoclonal antihuman SCF antibody (Amgen, Thousand Oaks, CA) conjugated to horseradish peroxidase (HRP) was added to all wells followed by a 1-hour incubation and washing. Substrate was then added to all wells and absorbances read with a microtiter plate reader. A standard curve was generated and unknown concentrations determined from the standard curve.

RESULTS

Concentration of SCF in peripheral blood serum. Using an enzyme immunoassay, the concentration of SCF was determined in 128 serum samples from 32 patients with AA. For all patients, samples were collected at presentation and at various time intervals after treatment with ALG (n = 26) or allogeneic BMT (n = 6). The results are presented in
Fig 1. SCF levels in peripheral blood serum. (II) Patients treated with ALG; (III) patients who underwent BMT. For each patient, the first bar represents the pretreatment serum levels. The following bars show posttreatment levels, with the time of sampling being indicated above the bar. A short follow-up means that the patient died early (Table 1). Patients who died are indicated by circled numbers. The normal values ($n = 267$) are given on the right; the mean normal value of 3.3 $\pm$ 1.0 ng SCF/mL is presented as a horizontal gray area and the minimum and the maximum normal levels of 1.3 and 8.0 ng/mL, respectively, are indicated as lines.

Fig 1. The patients, numbered 1 through 32, are arranged according to decreasing serum SCF levels and taking into account an average value of all determinations per patient. The normal range, as determined for 267 healthy blood donors, was $3.3 \pm 1.0$ ng/mL (mean $\pm$ SD; minimum and maximum values of 1.3 and 8.0 ng/mL, respectively) and is indicated as a gray horizontal area in Fig 1.

All 32 patients were diagnosed at presentation as having severe AA; their peripheral blood counts were low (Table 1) and the colony-forming capacity of their BM cells in vitro was virtually zero (results not shown). Yet, the pretreatment SCF serum levels varied widely from 0.33 to 6.1 ng/mL among individual patients. After treatment with either ALG or BMT, there was no recognizable pattern of changes; increased and decreased SCF serum levels were observed with similar frequency. However, despite this wide range, SCF concentrations in AA sera tended to be low: 26 of 128 sera contained less than 1.3 ng SCF/mL (lowest level of normal) and the level was below $3.3 \pm 1.0$ ng/mL (mean range of normal) in 74 samples. In only 21 of 128 sera, SCF was $\geq 3.3$ ng/mL. Statistical analysis by an unpaired $t$-test and the Mann-Whitney $u$-test comparing 267 normal and 128 aplastic samples showed a highly significant difference between SCF levels in these two groups of sera ($P = .0001$). Considering an average SCF concentration determined per patient, half of the patients (1 to 15) were within the normal mean values of $3.3 \pm 1.0$ ng/mL, whereas the other half (16 to 32) were below this range. Notably, a subgroup of patients (1 to 5) showed consistently high SCF serum levels and another subgroup (27 to 32) consistently low SCF serum levels regardless of hematopoietic function.

There was never a correlation between peripheral blood counts and SCF levels at any of the time points irrespective of the treatment. Strikingly, even after BMT, previously low SCF levels did not normalize, nor did previously high SCF lower after either BMT or ALG treatment. Considering that responding patients reconstitute BM function within a year after ALG treatment, we compared peripheral blood counts from this recovery period with SCF levels. Patients with low SCF serum concentrations tended to have lower blood values; however, this difference was not statistically significant.

A comparison of SCF levels with clinical parameters is of interest. Among 32 patients included in the study, 13 have died (Table 1, Fig 1). The survival rate was higher in patients 1 to 15, whose average SCF concentration was within the normal mean range, than in patients 16 to 32 having SCF levels below this range. Moreover, recovery of hematopoietic function in patients 1 to 5 with relatively high SCF
concentrations (3.3 ng/mL or higher) was very rapid, transfusion dependence lasted for no longer than 1 month post-treatment, and all five patients are currently alive and well after 3 to 8 years. On the other hand, the clinical course was poor in patients 27 to 32, who had very low SCF levels: of the ALG-treated patients, two died and never became transfusion independent. Only two of these patients are alive to date: one (patient 29), currently in remission, required transfusions for 9 months after therapy and one (patient 30) has suffered several relapses of aplasia and is now pancytopenic. Both BMT patients with low SCF values have died; one of graft rejection and one of graft-versus-host disease.

SCF level in BM plasma. Because BM stroma is considered the main cellular source of SCF, we measured SCF concentration in the BM plasma and compared it with the SCF concentration in serum in a group of 19 patients with AA (Fig 2). Results show a high ($R = .94$) correlation between both values in all patients, which indicates that measurement of SCF levels in peripheral blood serum reflect well the production of SCF by BM stroma.

DISCUSSION

The mechanism of functional changes in the hematopoietic microenvironment contributing to BM failure in AA remains unclear. Earlier reports demonstrated a frequent reduction of in vitro growth and hematopoietic activity of AA marrow stroma cultures. However, according to recent studies, stromal functions, including production of growth factors, such as G-CSF, GM-CSF, and IL-6, are normal in the majority of cultures from patients with AA. Concomitant expression of SCF in the pathophysiology of AA. Constitutive expression of SCF results in nanogram per milliliter serum concentrations, significantly higher than of the inducible growth factors. However, the particular property of SCF to synergize with several other hematopoietic growth factors may require the maintenance of high-threshold concentration of SCF. Our results suggest that in some patients with AA, deficiency in production of SCF may occur. These data support our conclusions from a previous study that demonstrated that the proliferation defect of aplastic cells can be partly corrected in vitro with SCF, which suggests an inadequate in vivo supply of the factor. Highly diverse SCF levels in individual patients may reflect heterogeneity in the pathophysiology of AA. However, there is no evidence that AA associated with low SCF expression is due to defects that would resemble stromal defects in murine S1 mutants having anemia caused by mutations at the SCF gene locus. To date, no human hematopoietic disorder has been found associated with genomic abnormalities affecting SCF or its receptor c-kit. However, it should be noted that measurements of factor levels in serum include only the secreted form of SCF. Studies on expression of the other biologically active, membrane-bound form of SCF are required to assess fully the role of SCF in the pathophysiology of AA.

Of interest is a comparison between SCF serum levels and clinical observations. Survival was higher and the duration of transfusion requirements after therapy shorter in a group of patients with high as opposed to patients with low SCF levels. Interpretation of these observations should, however,
be very cautious and consider that some of the patients underw ent BMT and hence, compared with ALG-treated patients, have a different mechanism of hematopoietic recovery. The number of patients in our study was too small for extended statistical analysis. Nevertheless, it is conceivable that high systemic levels of SCF have contributed to patient clinical improvement after either type of therapy. A similar conclusion has recently been drawn from results of analysis of SCF serum levels in three patients with Diamond-Blackfan anemia. Both observations reinforce the notion of a potential therapeutic value for SCF in human hypoproliferative disorders of hematopoiesis.

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