ERYTHROPOIESIS AFTER NONMYELOABLATIVE STEM-CELL TRANSPLANTATION IS NOT IMPAIRED BY INADEQUATE ERYTHROPOIETIN PRODUCTION AS OBSERVED AFTER CONVENTIONAL ALLOGENIC TRANSPLANTATION

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Background. It is now well established that after conventional allogeneic hematopoietic stem-cell transplantation (HSCT), erythropoietic recovery is impaired because erythropoietin (Epo) production remains inadequate for prolonged periods of time. However, erythropoietic reconstitution after nonmyeloablative SCT (NMSCT) has never been characterized.

Methods. Twelve patients received a nonmyeloablative conditioning regimen consisting of 2 Gy total body irradiation (TBI) alone (n=6), 2 Gy TBI and fludarabine (n=3), or cyclophosphamide and fludarabine (n=3), followed by transplantation of allogeneic peripheral blood stem cells. Graft-versus-host-disease (GvHD) prophylaxis was carried out with mycophenolate mofetil (from day −1 to day 28) plus cyclosporine (from day −1 to day 120 or longer in case of chronic GvHD). Erythropoiesis was quantitated by soluble transferrin receptor (sTfR) levels, and the adequacy of Epo production was evaluated by the observed-to-predicted Epo ratio (O/P Epo).

Results. Mean sTfR levels decreased following the conditioning regimen but remained well within the normal range throughout the posttransplant period. The O/P Epo ratio presented an initial surge quite similar to that observed after conventional conditioning. Thereafter, the O/P Epo ratio normalized rapidly, and Epo levels remained adequate during the whole observation period.

Conclusion. Contrarily to what is observed after myeloablative transplant, Epo levels remained adequate after NMSCT, resulting in normal erythropoiesis. These results suggest that the administration of erythropoietin therapy (rHuEpo) could be less effective after NMSCT than after conventional allogeneic transplant.

Allogeneic hematopoietic stem-cell transplantation (HSCT) is an effective treatment for selected hematologic malignancies (1, 2). Its curative potential is in part achieved through an immune-mediated destruction of malignant cells by donor lymphocytes termed the graft-versus-leukemia (GVL) effect (2–7). However, because of its toxicity, conventional allogeneic HSCT is restricted to younger and fitter patients (8). Therefore, several groups have developed the concept of nonmyeloablative stem-cell transplantation (NMSCT), in which the main mechanism of tumor eradica-

tion is shifted from high-dose cytotoxic agents to the GVL effect (9, 10). After extensive preclinical studies (11–15), the Seattle team developed a nonmyeloablative HSCT approach combining 2 Gy total body irradiation (TBI)±90 mg/m² fludarabine as a conditioning regimen and postgrafting immunosuppression with cyclosporine (CaA) and mycophenolate mofetil (MMF). This approach was recently shown to be feasible even in elderly or sick patients who were ineligible for a conventional transplant (9, 16). Because of the mild conditioning regimen given and the use of peripheral blood stem cells (PBSC) as the source of hematopoietic stem cells, posttransplant myelosuppression remained modest and transient (9, 16). Moreover, the Seattle team recently demonstrated that both red-blood-cell (RBC) and platelet transfusion requirements were reduced in NMSCT compared with conventional PBSC transplant recipients (10).

Erythropoetin (Epo) is the critical regulatory factor of erythropoiesis (17). In patients with normal kidney function, serum Epo levels increase exponentially when an anemia develops. After high-dose chemotherapy, serum Epo levels first rapidly increase to disproportionately high levels for 1 to 3 weeks, with peak values usually observed in the first week after the conditioning regimen (18–27). However, after allogeneic SCT, the Epo response to anemia then generally becomes impaired, resulting in inappropriately low Epo levels for the degree of anemia and prolonged anemia (21, 24). This is specific for allogeneic transplants because serum Epo levels remain adequate throughout the posttransplant course in recipients of an autologous marrow or PBSC transplant (19, 20, 22, 23, 25–28). This defect in Epo production has been attributed to the use of cyclosporine (29), which does not affect the expression of the Epo gene but causes an inhibition of Epo secretion (30). However, other factors such as graft-versus-host disease (GvHD) (20, 24, 28, 31) or cytomegalovirus (CMV) infection (20, 28) may also contribute to it. On the basis of these findings, it is not surprising that erythropoietin therapy (rHuEpo) shows some efficacy in the early posttransplant period after allogeneic (32, 33) but not autologous transplantation (32, 33), but is remarkably efficient when it is started after day 35 postallogeic transplantation (34).

However, erythropoietic recovery after a nonmyeloablative transplant has not been well characterized, and it is not known whether rHuEpo could enhance erythroid function in this setting. In this study, we examined the reconstitution of erythropoiesis in 12 recipients of a nonmyeloablative transplant (NMSCT group, n=12). Results were compared with a group of conventional bone-marrow-transplant (BMT) recipients previously reported (BMT group, n=47) (25) and with a group of patients undergoing a standard myeloablative PBSC transplant (PBSC group, n=6).
PATIENTS AND METHODS

Patients and Donors

We studied 12 patients (Table 1) receiving a PBSC transplant after a nonmyeloablative conditioning regimen (16). Written informed consent was obtained from patients to draw blood samples for the study. Conditioning (Table 1) consisted of 2 Gy single dose TBI alone on day 0 (n=6). For patients not heavily pretreated or for unrelated transplants, TBI was combined with 30 mg/m²/day fludarabine and cyclophosphamide at 1 g/m² per day for 3 days (Fluda-Cy) because they had previously received 12 Gy TBI as conditioning regimen for an autotransplant (Table 1). Posttransplant immunosuppression was carried out orally with cyclosporine (CsA) (CyA) and platelet transfusion were 8.0 g/dL and 15×10⁹/L, respectively. Granulocyte (G)-CSF (5 μg/kg per day) was administered when the granulocyte count was below 1.0×10⁹/L. The diagnosis and grading of acute and chronic GvHD was established as previously reported (16). Polymerase chain reaction (PCR) for CMV was performed weekly until day 100 and every 2 to 4 weeks thereafter. Patients with a positive PCR received preemptive ganciclovir for a minimum of 4 weeks and generally up to day 100.

Laboratory Analyses

CsA levels were measured by chromatography. Complete blood counts were determined in a Technicon H2 cell counter (Bayer, Tarrytown, NJ). Serum Epo levels were measured by a commercially available radioimmunoassay (Incstar Corp., Stillwater, MN). On the basis of regression equations obtained in appropriate reference subjects between hematoctrit (Hct) on the one hand and log (Epo) on the other, predicted log (Epo) values were derived for each Hct, and O/P ratios of observed–predicted Epo values were calculated (31). The mean (+ standard deviation) Epo O/P ratio in a cohort of 31 normal donors was 1.03±0.08. Serum-soluble transferrin receptor (sTfR), a quantitative measure of total erythropoietic activity, was measured by a commercially available ELISA (R&D, Minneapolis, MN). Normal values range from 3,000 to 7,000 µg/L.

Statistical Analysis

Unpaired Student’s t tests were used to compare biologic variables in two groups. Welch’s correction was used in case of unequal variance. The probability of GvHD and CMV reactivation were studied by life-table analyses, and Wilcoxon rank tests were used for comparisons between groups. Statistical analyses were carried out with Graphpad Prism (Graphpad Software, San Diego, CA).

RESULTS

Clinical Data

Mean CsA blood levels were 416±143 ng/mL on day 14, 308±116 ng/mL on day 42, and 208±66 ng/mL on day 100, respectively (Fig. 1). The actuarial 150-day incidence of grade I-IV (II-IV) acute GvHD was 41% (25). The actuarial 150-day incidence of CMV reactivation was 58%.

![Figure 1. Cyclosporine blood levels in the nonmyeloablative stem-cell transplantation (NMSCT) group. HSCT, hematopoietic stem cell transplantation.](image-url)
Erythropoietic Activity

NMSCT recipients were compared with conventional BMT recipients. There was little difference in Hemoglobin (Hb) levels after transplantation because patients in the BMT group were supported by RBC transfusions when their Hb fell below 10 g/dL (many transfusions), but patients in the NMSCT group maintained better Hb levels, with few transfusions when the Hb was less than 8 g/dL (Fig. 2). After conventional BMT, sTfR levels decreased sharply after the conditioning regimen to a minimum of 2040 ± 740/L on day 14 then increased up to day 60 but decreased again thereafter (P < 0.02 between days 60 and 150) (Fig. 3). The evolution was very similar in the conventional PBSC group with a nadir of 1000 ± 350 µg/L on day 14 followed by a recovery through day 80 and a secondary decrease thereafter. In the NMSCT group, sTfR levels were 6520 ± 2120 µg/L before conditioning and decreased significantly (P = 0.007) to a minimum of 4000 ± 1710 µg/L on day 10 but remained consistently above 5000 µg/L thereafter (Fig. 3). The initial sTfR decrement was significantly smaller than after conventional HSCT, and sTfR values never fell below the normal range.

Serum Erythropoietin Levels

After NMSCT, serum Epo levels peaked on day 0 with a mean O/P Epo of 1.12 ± 0.11 (P = 0.0057 compared with O/P Epo in 31 healthy donors), which was very similar to the O/P Epo of 1.11 ± 0.20 after conventional BMT (Fig. 4). Although O/P Epo after conventional BMT between days 14 and 180 were significantly lower than in normal subjects (P < 0.05 to P < 0.001) and significantly decreased compared with pre-transplant values (P < 0.02 to P < 0.001), Epo production was adequate throughout the posttransplant course after NMSCT (Fig. 4). Moreover, O/P Epo ratio remained significantly higher in the NMSCT group compared with the BMT group from day 28 to day 180 (Fig. 4). Indeed, the average O/P Epo of all samples obtained between days 14 and 180 in all NMSCT recipients (0.99 ± 0.19, n = 105) did not significantly differ from the O/P Epo of normal subject (1.03 ± 0.08, n = 31). Although the number of observations after conventional PBSC transplant was too low for differences to reach statistical significance at any particular time point, pooled O/P Epo values for the entire posttransplant course were significantly lower than in the NMSCT group (P = 0.0121).

**DISCUSSION**

Elevated serum Epo levels are observed transiently after intensive conditioning regimens without concomitant changes in Hb (18-27, 35, 36). The peak Epo values are
usually observed 0 to 7 days after transplant, at the time of the nadir of erythropoietic activity. There is now substantial evidence that serum Epo levels partly depend on the rate of Epo use by Epo receptor-bearing erythroid precursors (27, 37). Therefore, severe myelosuppression following the conditioning regimen could disrupt the usual Epo degradation pathway and provoke a surge of serum Epo concentration through prolonged Epo life span (27). However, the NMSCT recipients in the current study received a much milder conditioning regimen and yet still experienced a significant drop in erythropoietic activity (Fig. 3), resulting in some decrease in Hb levels (Fig. 2). Although the level of erythropoiesis remained well within the normal range, the surge in serum Epo levels was quite similar to that observed after myeloablative conditioning (Fig. 4). Indeed, it has been suggested that such changes in serum Epo levels could relate to change in the rate of production rather than in the rate of use (38).

With marrow recovery after a myeloablative transplant, Epo levels progressively return to an appropriate range, and the duration of this correction phase inversely correlates with the speed of engraftment (27). This is consistent with increased Epo consumption by an expanding pool of erythroid precursors. However, animal experiments indicate that marrow accumulation of Epo is minimal, and that Epo life span is not significantly influenced by erythropoietic activity (39, 40). Indeed, experimental data suggest that variations in plasma Epo levels during periods of rapidly expanding erythropoiesis are the result of decreased production rather than enhanced use (38), but the precise mechanisms remain to be elucidated.

After marrow recovery, endogenous Epo remains appropriate for the degree of anemia in autologous transplant recipients (19, 20, 22, 23, 25, 26) but rapidly becomes inadequately low in patients receiving a myeloablative allogeneic transplant (19–21, 24–26). This translates into erythropoietic activity and Hb levels remaining quite low for several months (25). This defect in Epo production has been partly attributed to the use of CsA (29, 30), which does not affect the expression of the Epo gene but causes an inhibition of Epo secretion (30). However, other factors such as acute GvHD (20, 24, 25, 28) or CMV infection (20, 28) have also been involved. We have recently shown that starting rHuEpo around day 35 after conventional allogeneic HSCT resulted in major responses in more than 90% of the patients (34), demonstrating that rHuEpo substitution at the time of marrow recovery can accelerate erythropoietic reconstitution that would otherwise remain impaired because Epo levels remain inadequate for prolonged periods of time.

Interestingly, our NMSCT recipients did not experience inappropriately low Epo levels for the degree of anemia nor the subsequent decrease in erythropoietic activity. Rather, Epo O/P ratios and sTfR levels were constantly well within the normal range. The reasons for this discrepancy with conventional transplants remain unclear. The use of PBSC instead of marrow as the source of stem cells cannot be incriminated because the control group of conventional PBSC transplants behaved similarly to the conventional BMT recipients. NMSCT recipients received CsA at least to day 120 after the transplant, and blood CsA levels were quite high. This could indicate that CsA is not the primary cause for the inappropriately low Epo levels observed after conventional transplant. Similarly, the rate of CMV reactivation in our NMSCT patients (58%) was comparable with that observed in our conventional BMT recipients (50%) (25).

Acute GvHD has also been associated with the defect in Epo production. The incidence of acute GvHD was lower in our NMSCT patient (41%) compared with our BMT group (64%) (25). This is in accordance with the generally lower incidence and severity of acute GvHD observed after NMSCT (16). This relatively low incidence of acute GvHD may partly explain their appropriate levels of serum Epo. It has been suggested that the persistence of recipient hematopoietic or immune cells (mixed chimerism) after NMSCT could contribute to improve donor-versus-host tolerance (41). One could speculate that such tolerance toward host tissues would also apply to host Epo-producing cells either directly or through more limited production of inhibitory cytokines (42).

In conclusion, contrary to what is observed after conventional myeloablative HSCT, serum Epo levels remain adequate throughout the posttransplant course after NMSCT. These results suggest that the administration of rHuEpo could be less effective after NMSCT than after conventional allogeneic transplant.

REFERENCES