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Circulating hematopoietic progenitors are not altered in patients with post-transplant erythrocytosis

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Granulocyte/macrophage (GM), mixed colony and erythroid burst forming unit assays were performed in 9 post-transplant erythrocytosis (PTE) patients, 18 non-PTE kidney transplant recipients and 12 healthy volunteers. The number of GM precursors was lower in PTE patients than in normal subjects. This indicates that hematopoietic stem cell potential is not altered in PTE.

The pathogenesis of erythrocytosis, which occurs in up to 20% of patients after renal transplantation, is unclear. Possibilities include transplant rejection, transplant artery stenosis, hydronephrosis, resetting of the erythropoietin threshold, resolution of hyperparathyroidism, immunosuppressants, hepatic or native kidney erythropoietin hypersecretion). In this study, hematopoietic stem cell reserve was investigated by *in vitro* assays for circulating granulocyte-erythroid-monocyte-megakaryocyte (CFU-GEMM), granulocyte-monocyte colony (CFU-GM) and erythroid burst forming units (BFU-E).

The study group comprised 27 kidney transplant recipients aged 27-68 (mean 44) years. Nine PTE patients had had symptoms of hyperviscosity, relieved by phlebotomies, in the previous year with maximal hematocrit values 0.52-0.64 (mean 0.55). Renal artery stenosis was not specifically looked for, but there was no suspicion that any patient had this condition. The remaining 18 non-PTE patients had serum creatinine values < 200 $\mu\text{mol/L}$.¹ The number of rejection episodes, hemodialysis duration and time after transplantation did not differ significantly between the two groups (data not shown). Twelve healthy male volunteers (22-45 years, mean 36) served as a control group.

Blood samples were collected in the morning and used immediately for cell counts and cultures. Sera for erythropoietin were frozen until assayed (EPO ELISA kit, Boehringer, Mannheim, Germany). *In vitro* assay was assessed in a methylcellulose-using modified technique suggested by the manufacturer (Stem Cell Inc., Vancouver, Canada). Briefly, separated mononuclear cells (MNC) were plated in triplicate in two concentrations, 0.5 and 1×10^5 cells/mL of standardized mixture medium (MethoCult H4433, Stem Cell) containing 30% fetal bovine serum, 1% bovine serum albumin, 10^{-4} mol/L 2-mercaptoethanol, 2 mmol/L L-glutamine, 5% PHA-LCM in serum and 3 U/mL of human recombinant erythropoietin,² and

Table 1. Data (median and range) on indicators of hematoipoiesis for patients and controls. A significant difference ($p < 0.05$) between patients and controls is marked by an asterisk.

Indicator*°	Patients		Controls (n=12)
	with erythrocytosis (n=9)	w/o erythrocytosis (n=18)	
BFU-E	14 (4-85)	13 (1-41)	13 (3-27)
CFU-GM	7 (3-29)*	11 (2-37)	21 (9-37)
CFU-GEMM	1 (0-5)	2 (0-10)	3 (0-9)
Erythropoietin	199 (136-406)	146 (114-281)	–

*All committed progenitors were counted and expressed per 10^5 mononuclear cells: BFU-E, erythroid burst forming units, CFU-GM, granulocyte colony-forming units, CFU-GEMM, granulocyte-erythroid-monocyte-megakaryocyte colony forming units; °erythropoietin was expressed in IU/L.

scored without staining on day 14.^{2,3}

The differences in laboratory parameters between groups were analyzed using the Kruskal-Wallis non-parametric test. If the difference was significant, a Mann-Whitney test was performed to assess the difference between patients and controls.

Stem cell assays showed that only the CFU-GM colony number was significantly lower in PTE patients than in controls. (Table 1, $p < 0.05$). Erythropoietin in all patients was above the assay manufacturer's reference range (4-90 IU/L) with no difference between groups. Results were also evaluated according to the immunosuppressive therapy (cyclosporin and corticoids with or without azathioprine), but no differences in colony numbers or erythropoietin were found (not shown), except a lower blood leukocyte count in patients receiving triple therapy ($p < 0.05$, data not presented).

One PTE patient had an exceptionally high BFU-E count (85 colonies/ 10^5 MNC) at 227 $\mu\text{mol/L}$ of serum creatinine.¹

In normal hematopoiesis stem cells in bone marrow and peripheral blood are in a steady state, and presumed to be by 1-2 logarithms lower than those in the bone marrow.⁴ In PTE the erythroid progenitors may have increased proliferative capacity or could be more sensitive to erythropoietin.^{5,6} Our results indicate that circulating progenitors are not altered in patients after kidney transplantation, as there was no difference between patients' groups and controls. Normal CFU-E count with normal responsiveness to erythropoietin in PTE has been reported.⁷ This is at variance with findings in polycythemia vera.⁸ The stimulation of erythroid precursors may be achieved by cyclosporin,⁹ although this drug can impair endogenous erythropoietin production.¹⁰ Lower CFU-GM counts in patients after kidney transplantation, (significant only for PTE patients) might suggest disruption of differ-

entiation towards myeloid cell lineage, but further investigations are needed to prove this.

In conclusion, no alteration of circulating hematopoietic stem cell reserve or disruption in the early phases of erythropoiesis in PTE patients was observed.

Key words

Erythrocytosis, kidney transplantation

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