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Angiotensin-Converting Enzyme Inhibition Induces Apoptosis in Erythroid Precursors and Affects Insulin-Like Growth Factor-1 in Posttransplantation Erythrocytosis

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Abstract. A number of studies suggest that erythropoietin (Ep), angiotensin II, and insulin-like growth factor (IGF-1) are involved in the pathogenesis of posttransplantation erythrocytosis (PTE). Angiotensin-converting enzyme inhibitors (ACEI) are the treatment of choice in PTE, but their mechanism of action is unclear. It was shown previously that ACEI added directly to cultures of erythroid precursors from patients with PTE inhibit colony growth. In this report, the effect of ACEI on CD34+ erythroid precursor apoptosis was studied, as were hematocrit (Hct), Ep, IGF-1, and IGF-binding protein 3 (IGF-BP3) levels. Ten patients with PTE, 10 transplant control patients, and 7 normal control subjects were studied. Peripheral blood CD34+ cells were isolated, and apoptosis was assessed by annexin assay and DNA laddering before and during ACEI therapy. At the same time, Hct, Ep, IGF-1, and IGF-BP3 levels were measured. Baseline CD34+ cell number, CD34+ apoptosis, Ep, IGF-1, and IGF-BP3 levels were the same between PTE and transplant control subjects. ACEI therapy was associated with a striking increase in CD34+ cell apoptosis and a decrease in Hct in both groups. In contrast to control subjects, patients with PTE on ACEI showed a significant decrease in IGF-1 levels and a greater percentage decrease in Hct. In normal control subjects, ACEI therapy was associated with a fall in Hct but no change in CD34+ cell apoptosis. In PTE, ACEI-related increase in erythroid progenitor apoptosis may partially explain the ACEI-associated decrease in Hct. However, it is not clear that erythroid precursor apoptosis is related to changes in IGF-1 or IGF-BP3.

The cause of erythrocytosis after renal transplantation is unknown, but a number of studies suggest that erythropoietin (Ep), angiotensin II (AII), and insulin-like growth factor-1 (IGF-1) and its binding proteins may be involved. Increased Ep production has been implicated in the pathogenesis (1,2), yet many patients with posttransplantation erythrocytosis (PTE) have low or even undetectable Ep levels (2,3). We showed recently that cultures of erythroid precursors (burst-forming units–erythroid [BFU-E]) from patients with PTE are more sensitive to low concentrations of Ep than those from transplant control subjects (4). AII, via its type 1 (AT_1) receptor, is an important growth factor for a number of cell types (5–7), and AII has been shown to stimulate the growth of BFU-E from normal individuals (8). The use of an angiotensin-converting enzyme inhibitor (ACEI) or a selective AT_1 receptor antagonist causes a decrease in hematocrit (Hct) by decreasing red cell production in patients with PTE (2,9,10). We showed that ACEI, added directly to cultures of BFU-E from patients with PTE, inhibits colony growth (4). These observations strengthen the concept that AII is important in the pathogenesis of PTE.

ACEI also may affect IGF-1 levels in renal transplant recipients. Several groups have reported that renal transplant patients with or without PTE have increased serum concentrations of IGF-1 (11) or IGF-1 binding proteins (12) when compared with control subjects. Patients who had PTE and were treated with ACEI to normalize their Hct showed a decrease in IGF-1 levels compared with pretreatment values (11). These data suggest a potential role for IGF-1 and its binding proteins in PTE.

ACE inhibition promotes apoptosis in vascular smooth-muscle cells (13,14). In contrast, Ep, AII, and IGF-1 are known to have antiapoptotic or growth-promoting effects in erythroid progenitor cells (15–17). However, the effect of ACEI on apoptosis of erythroid precursors has not been studied. In the present study, we studied the effect of ACEI use on apoptosis of CD34+ erythroid precursors and on changes in Hct, IGF-1, and IGF-binding protein 3 (IGF-BP3) levels in patients with PTE, transplant control patients, and normal control subjects.

Materials and Methods

Patients

We studied 20 renal transplant patients who had stable renal function 6 mo or more posttransplantation. Ten had PTE, defined as Hct 51% or greater on at least three consecutive measurements and increased red cell

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mass. Other causes for erythrocytosis were excluded by clinical and laboratory testing, as described previously (4). The other 10 were transplant patients matched for gender, age, serum creatinine, transplant duration, and maintenance immunosuppression. The control patients had normal Hct that never exceeded 47% posttransplantation. All patients had retained native kidneys. Except for one control patient, all patients had received only one transplant. Five PTE and two control patients had living donors. Only two patients with PTE had been treated previously with ACEI, and this therapy had been discontinued 3 mo before our study. Thus, no patient was on ACEI or AT_1 blocker therapy at the beginning of the study. With the exception of a decrease in patients' calcium channel blocker therapy, as clinically indicated, all other medication was kept constant during the study. We also studied seven normal male subjects who were on no medications and who had normal BP, urinalysis, renal function, liver function, and complete blood count. The study protocol was approved by the Institutional Review Board of Montefiore Medical Center, and all patients gave written informed consent before entering the study.

ACEI Therapy

Lisinopril or fosinopril (9.25 ± 1.8 mg/d; range, 5 to 10 mg/d) was begun after baseline studies were obtained. The ACEI therapy was titrated to BP and was maintained for at least 3 wk until the patients were available for the follow-up studies. In the 20 renal transplant patients, mean duration of ACEI therapy was 6.8 ± 4.6 wk. The seven normal subjects each took fosinopril 10 mg/d for 4 wk.

Isolation of CD34+ Erythroid Precursors

Circulating erythroid progenitor cells were isolated as described previously (18). Eighty milliliters of heparinized peripheral venous blood was obtained at least twice from each patient. The blood was diluted 1:1 with α-minimal essential medium without fetal calf serum, and the low-density mononuclear cells (1.077 g/ml) were isolated by centrifugation (1300 rpm for 30 min) over Ficoll-Hypaque. The low-density cells then were labeled with magnetic particles by use of a cocktail of tetrameric antibody complex, as recommended by the manufacturer (Stem Cell Technologies, Inc., Vancouver, British Columbia, Canada). The lineage-negative CD34+ cells were collected, and the magnetically labeled lineage-positive cells remained bound to the column. As demonstrated by fluorescence-activated cell sorter analysis, approximately 80% of CD34+ cells in the enriched fraction were BFU-E. The selected CD34+ cells were counted and either diluted for plating in the methylcellulose clonogenic assay or assayed for apoptosis by use of annexin staining.

Apoptosis Measurement by Annexin Assay

Annexin assay is based on the observation that soon after initiating apoptosis, most cell types translocate the plasma membrane phospholipid phosphatidylserine from the inner face of the plasma membrane to the cell surface. The surface phosphatidyl serine can be detected easily by immunofluorescence, by staining cells with a FITC conjugate of annexin V that has a strong natural affinity for phosphatidylserine. Externalization of phosphatidylserine is a stimulus-independent marker for apoptosis that precedes the nuclear changes (19). CD34+ cells were washed once with phosphate-buffered saline, incubated with annexin V–FITC for 15 min at room temperature in the dark, and visualized by fluorescent microscopy. The results are reported as the number of annexin-positive cells per 100 cells counted. The investigators who read this assay were blinded to clinical data from the patients.

Apoptosis Detection by DNA Laddering

As an additional assay for apoptosis, internucleosomal DNA fragmentation was detected by DNA laddering (20). Briefly, cells were pelleted, washed with cold phosphate-buffered saline, resuspended in 500 μl of lysis buffer (1% sodium dodecyl sulfate, 25 mM ethylenediaminetetraacetate, and 1 mg/ml proteinase K [pH 8]), and incubated overnight at 50°C. Ribonuclease A (10 mg/ml) then was added for an additional 2-h incubation at 37°C. The chromosomal DNA was extracted with phenol/chloroform, precipitated with ethanol, and analyzed by agarose gel electrophoresis, followed by staining with ethidium bromide to reveal the laddering pattern.

Clonogenic Assay

Colony-forming unit cultures were prepared in duplicate. Each 1 ml of methylcellulose (Methocult 4330; Stem Cell Technologies) was supplemented with 2 mM L-glutamine, 3 U/ml human erythropoietin (Stem Cell Technologies), 20 ng each of human interleukin-3 and granulocyte macrophage–colony stimulating factor (Stem Cell Technologies), and 50 ng human Steel Factor (purified in the Terry Fox Laboratory from supernatants of human Steel Factor c-DNA transfected COS cells). The cells were plated at three different concentrations (2.5, 5.0, and 10.0×10^2/ml), and each plate was scored after 14 to 18 d of incubation at 37°C in a humidified atmosphere of 5% CO_2 for the presence of erythroid (BFU-E–derived), granulocyte-macrophage (colony-forming unit–granulocyte macrophage–derived), and multilineage colonies (colony-forming unit–GEMM–derived) by use of standard criteria (18). The investigator who interpreted this assay was blinded to clinical data.

Laboratory Data

All blood tests were collected between 8 and 11 a.m. after an overnight fast. Complete blood counts were performed by use of a Sysmex SE 9000 (Toa Medical Electronics Co., Ltd., Kobe, Japan). Serum creatinine, lactic dehydrogenase, and total and indirect bilirubin levels were measured by autoanalyzer (Hitachi Medical Corp. of America, Tarrytown, NY). Erythropoietin levels were performed by chemiluminescence immunoassay (Nichols Institute Diagnostic, San Juan Capistrano, CA). IGF-1 levels were determined by RIA (Immunlite; DPC, Cirrus, Inc., Randolph, NJ). IGF-BP3 levels were performed by RIA (Endocrine Science, Calabasas Hill, CA). Specimens for IGF-1 and IGF-BP3 were collected at room temperature.

Statistical Analyses

Statistical analyses were performed by use of the SPSS software package (SPSS Inc., Chicago, IL). The unpaired *t* test was used to compare means between two groups, and the paired *t* test was used to compare data within the same group. ANOVA was used to compare results among three groups. Univariate correlations were evaluated by the Spearman test. The variables tested were Hct, apoptosis, IGF-1, IGF-BP3, Ep, age, duration of end-stage renal disease, transplant duration, presence of PTE, time of onset of PTE, duration of PTE, use of β blockers, calcium-channel blockers, diuretics, BP, weight, white blood cell count, platelet count, mean corpuscular volume, levels of lactic dehydrogenase, and total and indirect bilirubin. $P < 0.05$ was considered to indicate statistical significance. All data are presented as mean ± SD.

Results

Table 1 shows clinical and laboratory features of PTE and transplant control patients. There were no significant differences in age, gender, cause of end-stage renal disease, duration of

Table 1. Clinical and laboratory data: patients with PTE and transplant control patients[a]

Parameter	PTE ($n = 10$)	Transplant Control ($n = 10$)
Age (yr)	45.5 ± 15.7	41.8 ± 10.0
Gender	8 M, 2, F	9 M, 1 F
Cause of ESRD		
HTN	4	7
PCKD	2	—
DM	1	—
GN	2	2
other	1	1
ESRD duration (mo)	33.4 ± 19.9	48.6 ± 34.6
Immunosuppression		
PA	1	—
PAC	2	4
PCM	3	2
PT	2	2
PTM	2	2
Transplant duration (mo)	58.0 ± 68.6	51.9 ± 40.2
Onset of PTE (mo)	8.6 ± 2.7	—
Creatinine (mg/dl)	1.5 ± .5	1.3 ± .3

[a] PTE, posttransplantation erythrocytosis; ESRD, end-stage renal disease; HTN, hypertension; PCKD, polycystic kidney disease; DM, diabetes mellitus; GN, glomerulonephritis; P, prednisone; A, azathioprine; C, cyclosporine; M, mycophenolic acid mofetil; and T, tacrolimus.

end-stage renal disease, maintenance immunosuppression, transplant duration, or serum creatinine. The mean onset of PTE was 8.6 ± 2.7 mo (range, 2 to 30 mo). The mean duration of PTE at the time of the study was 45.8 ± 65.0 mo (range, 6 to 192 mo).

Table 2 shows baseline and treatment values of Hct and apoptosis of CD34+ erythroid precursors in patients with PTE, transplant control patients, and normal control subjects. As expected, the baseline Hct in patients with PTE was markedly higher than that in control groups ($P < 0.001$). Baseline apoptosis of CD34+ erythroid precursors was similar in the three groups. During ACEI therapy, Hct decreased significantly in patients with PTE (53.0 ± 1.8% *versus* 44.9 ± 5.3%; $P < 0.01$), transplant control patients (43.4 ± 2.3% *versus* 40.0 ± 3.0%; $P < 0.01$), and normal control subjects (44.2 ± 2.1% *versus* 41.8 ± 2.1%; $P < 0.01$). The mean duration of ACEI use in patients with PTE was 7.4 ± 3.8 wk and in transplant control patients was 6.3 ± 4.4 wk (P = NS). The percentage change in Hct with ACEI was significantly greater in patients with PTE than that in normal control subjects (−15.4 ± 7.8% *versus* −5.4 ± 3.4%; $P < 0.03$) and approached significance when patients with PTE were compared with transplant control patients (−15.4 ± 7.8% *versus* −7.6 ± 7.3%; $P < 0.055$). Apoptosis of CD34+ erythroid progenitor cells increased significantly in both patients with PTE (11.9 ± 6.3% to 60.3 ± 11.0%; $P < 0.001$) and transplant control patients (12.6 ± 6.2% to 59.6 ± 15.2%; $P < 0.001$) during ACEI therapy, but there was no difference in apoptosis between these two groups. Figures 1 and 2 show examples of positive annexin and DNA-laddering assays for apoptosis. In contrast, normal control subjects showed no significant change in apoptosis during ACEI therapy.

Table 2 and Figure 3 show the effects of ACEI therapy on IGF-1, IGF-BP3, and EP levels in patients with PTE, transplant control patients, and normal control subjects. There were no significant differences in baseline levels among the groups. During ACEI therapy, serum IGF-1 levels decreased significantly in patients with PTE (251.1 ± 145.9 to 207.1 ± 83.8 ng/ml; $P < 0.03$) and increased significantly in transplant control patients (276.7 ± 133.6 to 334.9 ± 161.4 ng/ml; $P < 0.003$) but did not change significantly in normal control subjects (189.5 ± 66.7 to 177.2 ± 40.5 ng/ml; P = NS). On ACEI therapy, serum IGF-1 levels were significantly greater in transplant control patients compared with normal control subjects (334.9 ± 161.4% *versus* 177.2 ± 40.5 ng/ml; $P < 0.05$). The percentage change of IGF-1 levels from baseline was significantly different between patients with PTE and transplant control patients (−13.3 ± 16.4% *versus* 18.1 ± 17.0%; $P < 0.001$). Levels of Ep and IGF-BP3 were not significantly changed during ACEI therapy. In the 10 patients with PTE, Ep levels increased in 5 and decreased in the other 5 during ACEI therapy. There was no difference in the IGF-1 levels between these two subgroups. There were no significant differences at baseline or during ACEI in white blood cell or platelet counts, mean corpuscular volume, lactic dehydrogenase, bilirubin, BP, body weight, or use of diuretics or calcium channel blockers between groups (data not shown). However, there was significant difference in β-blocker therapy between patients with PTE and transplant control patients (20 *versus* 70%; $P < 0.05$).

In renal transplant patients, ACEI caused an increase from baseline apoptosis in CD34+ cells within 2 to 3 wk (Figure 4). Maximal apoptosis was noted within 3 to 6 wk of therapy and was sustained in one patient who did not return for follow-up studies until 16 wk on ACEI therapy. Five patients (two PTE and three control) agreed to repeat apoptosis studies several weeks after they had discontinued ACEI. The ACEI-related apoptosis reverted to baseline values in most patients by 2 to 4 wk off medication. There was no significant difference in the number of BFU-E colonies between PTE ($n = 4$) and control ($n = 5$) groups before ACEI therapy (77.8 ± 48.5 *versus* 88.2 ± 67.6, P = NS).

Discussion

We made the novel observation that the use of ACEI enhanced apoptosis of CD34+ erythroid precursors isolated from peripheral blood of renal transplant recipients. Patients with PTE and transplant control patients had the same baseline apoptosis and the same marked increase in apoptosis during ACEI therapy. The time course of ACEI-related apoptosis was similar in the two groups. As erythroid precursor apoptosis increased, there was a concurrent significant decrease in Hct in both groups, which suggests that the decrease in Hct may be causally related to enhanced apoptosis. However, the fall in Hct was more pronounced in patients with PTE. In fact, we were unable to show a direct correlation between the change in

Table 2. Effect of ACEI therapy on apoptosis of CD34+ erythroid precursors and selected laboratory features in renal transplant patients and normal controls[a]

Parameter	Transplant Erythrocytosis ($n = 10$)	Transplant Controls ($n = 10$)	Normal Controls ($n = 7$)
Baseline			
hematocrit (%)	53.0 ± 1.8	43.4 ± 2.3[b]	44.2 ± 2.1[b]
apoptosis (%)	11.9 ± 6.3	12.5 ± 6.2	12.2 ± 10.2
Ep (mU/ml)	9.6 ± 9.6	10.6 ± 3.4	14.4 ± 3.9
IGF-1 (ng/ml)	281.1 ± 145.9	276.7 ± 133.6	189.5 ± 66.7
IGF-BP3 (mg/L)	2.9 ± 0.9	3.7 ± 0.8	3.5 ± 0.5
During ACEI			
hematocrit (%)	44.9 ± 5.3[c]	40.0 ± 3.0[d]	41.8 ± 2.1[d]
apoptosis (%)	60.3 ± 11.0[e]	59.6 ± 15.2[e]	12.4 ± 21.3
Ep (mU/ml)	11.5 ± 7.6	11.0 ± 11.1	22.8 ± 27.2
IGF-1 (ng/ml)	207.1 ± 83.8[f]	334.9 ± 161.4[g]	177.2 ± 40.5[h]
IGF-BP3 (mg/L)	2.8 ± 0.7	3.5 ± 1.0	4.2 ± 0.6
Δhematocrit (%)	−15.4 ± 7.8	−7.6 ± 7.3	−5.4 ± 3.4[i]
Δapoptosis (%)	550.1 ± 378.5	478.5 ± 216.2	166.6 ± 548.2
ΔEp (%)	310 ± 499.0	16.6 ± 115.8	38.5 ± 112.9
ΔIGF-1 (%)	−13.3 ± 16.4	18.1 ± 17.0[j]	−7.1 ± 61.2
ΔIGF-BP3 (%)	−8.3 ± 27.2	−1.8 ± 26.1	23.0 ± 32.5

[a] ACEI, angiotensin-converting enzyme inhibitor; Δ, percentage change from baseline; Ep, erythropoietin; IGF-1, insulin-like growth factor–1; IGF-BP3, IGF–binding protein 3; PTE, posttransplant erythrocytosis.
[b] $P < 0.001$ PTE *versus* transplant and normal controls.
[c] $P < 0.001$ PTE baseline *versus* ACEI.
[d] $P < 0.01$ transplant and normal controls baseline *versus* ACEI.
[e] $P < 0.001$ baseline *versus* ACEI.
[f] $P < 0.03$ baseline *versus* ACEI.
[g] $P < 0.003$ baseline *versus* ACEI.
[h] $P < 0.05$ transplant control *versus* normal control.
[i] $P < 0.03$ PTE *versus* normal control.
[j] $P < 0.05$ PTE *versus* transplant control.

apoptosis and the change in Hct. Thus, the ACEI-related increase in erythroid progenitor apoptosis may explain only partially the ACEI-mediated decrease in Hct. Also, a decrease in precursor cell apoptosis does not seem to explain the pathogenesis of PTE.

In normal control subjects, 4 wk of ACEI therapy led to a significant drop in Hct without a concurrent increase in erythroid precursor apoptosis, which suggests that the concurrent use of medication such as immunosuppressives or other factors may be required to affect apoptosis. Because there was no correlation between the fall in Hct and the change in Ep, IGF-1, or IGF-BP3 in normal control subjects, the ACEI-related drop in Hct remains unexplained.

Our data suggest that ACEI-related changes in IGF-1 also may be important in erythropoiesis in renal transplant recipients. With ACEI treatment, there were striking changes in IGF-1 levels in both patients with PTE and transplant control patients. In patients with PTE, IGF-1 levels decreased significantly and univariate analysis showed a strong correlation between the percentage decrease in Hct and the percentage decrease in IGF-1. These results are very similar to a recent report (11). Conversely, in transplant control patients, IGF-1 levels increased significantly with ACEI treatment. We do not have an explanation for the discordance in IGF-1 levels in patients with PTE and transplant control patients on ACEI therapy. We speculate that the differences that we observed in IGF-1 response to ACEI therapy between patients with PTE and transplant control patients are consistent with an abnormality in IGF-1 and IGF-1–binding protein regulation in PTE, as suggested by Brox *et al.* (12). However, it is not clear that the CD34+ erythroid precursor apoptosis that we observed was related to changes in IGF-1 or IGF-BP3.

Previous studies showed that IGF-1 is an important erythropoietic growth factor (17,21–25). *In vitro* studies of murine and human erythroid precursors have shown that IGF-1 inhibited apoptosis (17) and stimulated cell growth to maximal levels in the presence of suboptimal Ep concentrations (17,25) and that human BFU-E have receptors for IGF-1 (21). In a murine chronic renal failure model, the combination of low-dose Ep and IGF-1 was more effective in reversing anemia than either treatment alone (24). Two anephric patients who had normal circulating levels of hemoglobin but no measurable Ep and in whom IGF-1 was believed to be the major erythropoietic growth factor have been described (22,23). In eight

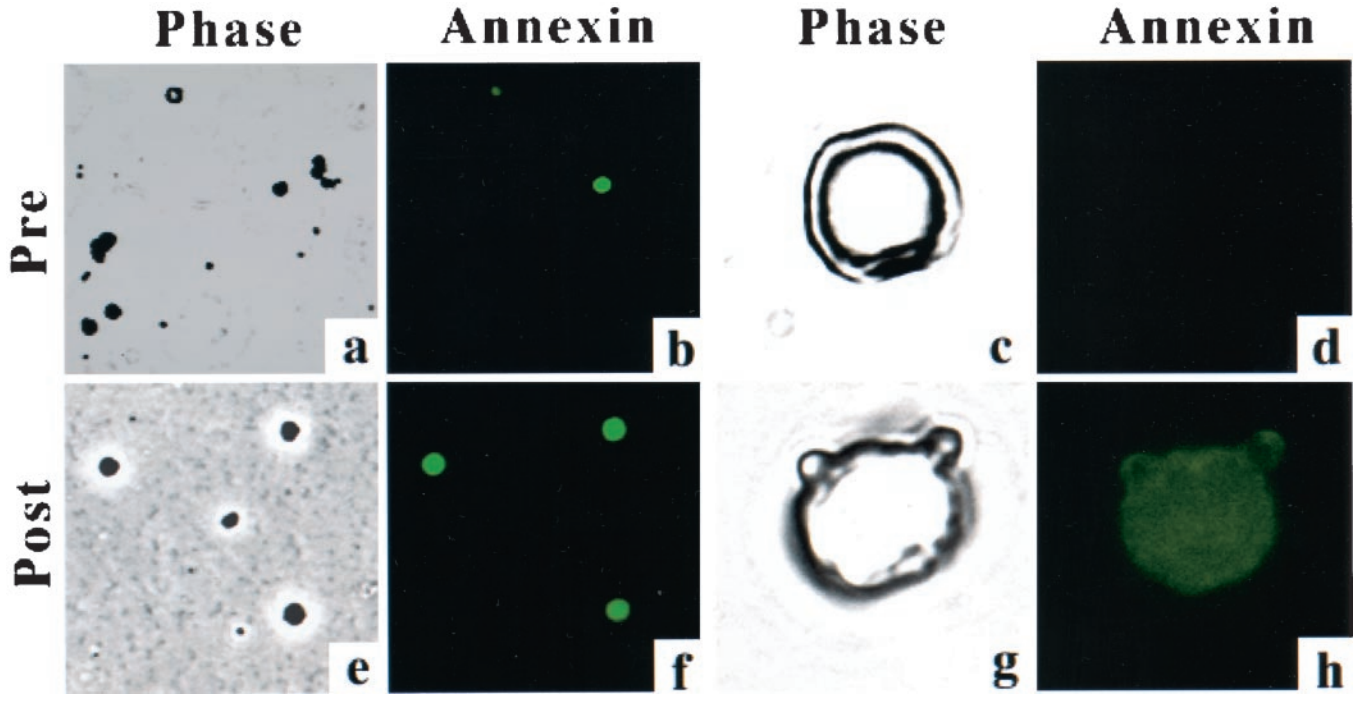

Figure 1. Angiotensin-converting enzyme inhibitor (ACEI) therapy induces apoptosis of CD34+ erythrocyte precursors—annexin assay. (a) Cells under low-power (10×) phase microscopy. (b) The same cells as in (a), stained with annexin. Note that only two of approximately eight cells in this field are annexin positive. (c) Normal CD34+ cell under high-power (100×) phase microscopy. (d) The same cell stained with annexin. Note the normal rounded morphology and absence of annexin staining, which indicates a nonapoptotic cell. (e) Cells after ACEI therapy, under low-power phase microscopy. (f) The same cells as in (e), stained with annexin. Note that three of four cells in this field are annexin positive. (g) Cell after ACEI therapy under high-power phase microscopy. (h) The same cell stained with annexin. Note the abnormal morphology, including membrane blebbing, and intense annexin staining, which indicates an apoptotic cell.

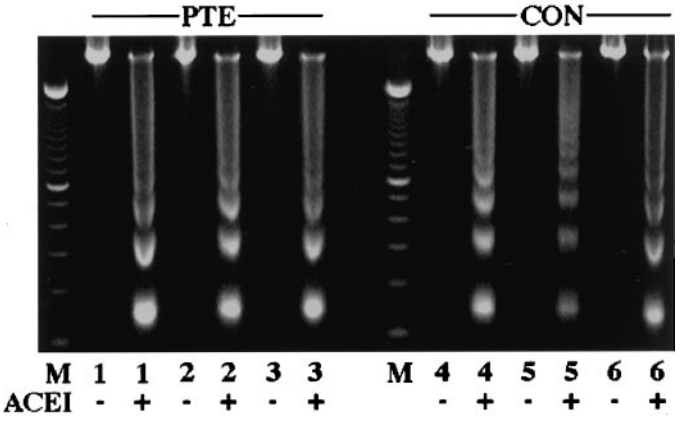

Figure 2. ACE inhibitor therapy induces apoptosis of CD 34+ erythrocyte precursors—DNA fragmentation assay. ACE inhibition resulted in a DNA laddering pattern characteristic of apoptosis, which confirms the results obtained by annexin of the same samples. ●, posttransplantation erythrocytosis (PTE); ○, control.

patients who were undergoing hemodialysis and had erythrocytosis, IGF-1 levels were significantly higher than those in normal control subjects and were inversely correlated with Ep levels. Antibody to IGF-1 partially blocked the stimulating effect of serum from the patients on normal erythroid precursors in culture (26). In another study of uremic patients with severe anemia and secondary hyperparathyroidism, IGF-1 levels were significantly correlated with Hct levels (27). Thus, IGF-1 could be an important factor in the regulation of erythropoiesis in patients with chronic renal failure.

Several studies have evaluated the role of IGF-1 in PTE. Eighteen patients who had PTE and were studied for 6 wk after ACEI withdrawal showed no change in IGF-1 levels, Ep, renin, or AII, despite a significant increase in Hct (28). A more recent study showed that 20 patients with PTE had higher Ep and IGF-1 levels than either transplant control patients or normal control subjects. ACEI therapy in the PTE group led to a decrease in Hct, Ep, and IGF-1 levels, and there was a significant correlation between Hct and IGF-1 (11). The effect of ACEI on transplant control patients was not assessed. Another recent report found that 18 patients with PTE had elevated IGF–binding protein 1 and 3 levels, compared with transplant control patients (12). However, there was no difference in IGF-1 and Ep levels. The effect of ACEI on IGF-1 or binding protein levels was not studied. Differences between these previous studies and the present study may be due to patient selection or variable type, dosage, and duration of ACEI therapy.

The role of Ep in PTE is unresolved. Studies have implicated increased Ep production as the cause of PTE (1,2,11,29). The Hct-lowering effect of ACEI and AT_1 blockers has been attributed to decreased Ep production, as demonstrated by decreased Ep levels (9,11,30,31). However, other studies have noted that patients with PTE often have low or undetectable Ep

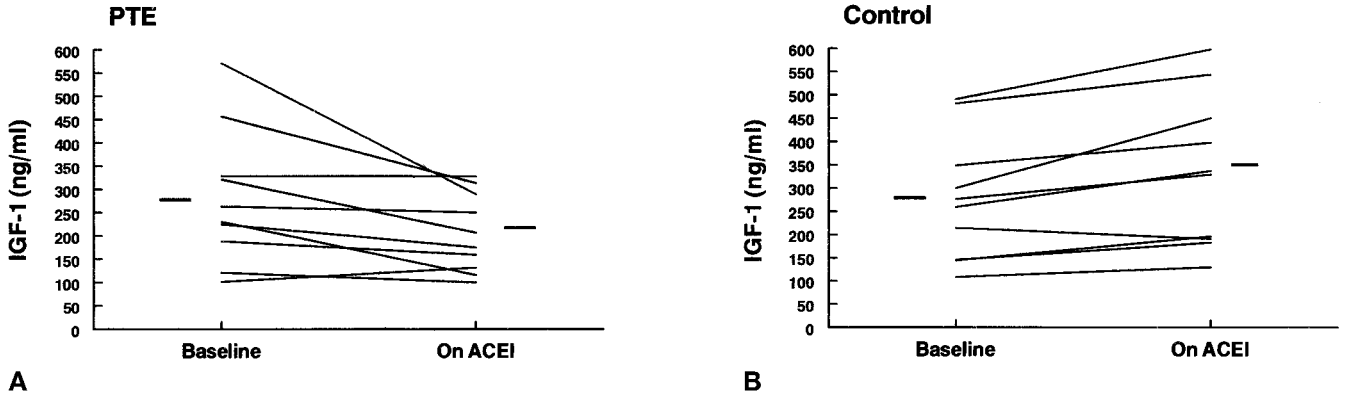

Figure 3. Effects of ACEI therapy on insulin-like growth factor-1 (IGF-1) levels in patients with PTE (A) and transplant control patients (B). On ACEI, there was a significant decrease in IGF-1 levels in the PTE group (281.1 ± 145.9 to 207.1 ± 83.08; $P < 0.03$). In the control group, ACEI therapy was associated with a significant increase in IGF-1 levels (276.7 ± 133.6 to 334.9 ± 161.4; $P < 0.003$).

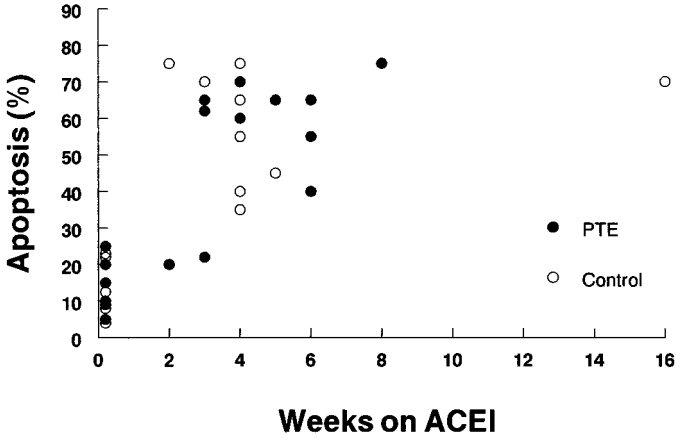

Figure 4. Time course of ACEI-mediated CD34+ cell apoptosis. ACEI therapy caused an increase in apoptosis within 2 to 3 wk. Maximal apoptosis seemed to occur within 3 to 6 wk of therapy. Each dot represents one apoptosis assay. Each patient had at least two measurements.

levels (2,3,28,32) and that ACEI and AT_1 blockers therapy may lead to a lower Hct without changes in Ep (3,32,33). In the present study, there was no difference in Ep levels between patients with PTE and transplant control patients before or during ACEI therapy. During ACEI treatment, we were unable to confirm any strong correlation between Ep or change in Ep and Hct, IGF-1, IGF-BP3, or apoptosis.

A number of drugs commonly used in the treatment of renal transplant patients may affect apoptosis of mesangial cells, renal tubule cells, and vascular smooth-muscle cells, including cyclosporine, thiazide diuretics, ACEI, β blockers, and calcium-channel blockers (13,14,34–36). Drug combinations may have a synergistic action on apoptosis. An example of this is the greatly enhanced apoptosis of cultured human vascular smooth-muscle cells in the presence of captopril and propranolol (14). The direct effects of these agents on erythroid progenitor cells are unknown. However, given that these drugs affect the renin-angiotensin system, it is possible that they all may have indirect influence on erythroid growth factors such as AII. In our study, β-blocker therapy was more prevalent in the transplant control patients than in the patient with PTE, although the use of cyclosporine, tacrolimus, thiazide, ACEI, and calcium-channel blockers was no different. We cannot exclude the possibility that β blockers increased low levels of apoptosis in controls to higher levels comparable to patients with PTE.

Erythropoiesis is a complex process that involves the interplay of multiple cytokines, growth factors, and growth inhibitors (36). Secondary erythrocytosis generally is caused by a disturbance in one or more regulatory factors. Although the pathogenesis of PTE remains unclear, recent studies have focused on the potential role of Ep, IGF-1, and AII, growth factors for erythroid progenitor cells with common signal transduction pathways (8,36,37). Our data suggest that in renal transplant recipients, ACEI affects at least two of these three factors, leading to decreased erythroid progenitor growth and increased apoptosis. These ACEI-mediated changes are most striking in patients with PTE. The mechanism of ACEI-related apoptosis in CD34+ erythroid precursors is unknown. We hypothesize that the enhanced sensitivity to ACEI in patients with PTE may be due to differences in cell cycling and differences in growth factor receptor expression. It will be important, in future studies, to test these possibilities.

References

1. Thevenod F, Radtke HW, Grutzmacher P, Vincent E, Koch KM, Schoeppe W, Fassbinder W: Deficient feedback regulation of erythropoiesis in kidney transplant patients with polycythemia. *Kidney Int* 34: 227–232, 1983
2. Gaston RS, Julian BA, Curtis JJ: Post transplant erythrocytosis: An enigma revisited. *Am J Kidney Dis* 25: 1–11, 1994
3. Danovitch GM, Jamgotchian NJ, Eggena PH, Paul W, Barrett JD, Wilkinson A, Lee DBN: Angiotensin converting enzyme inhibition in the treatment of renal transplant erythrocytosis. *Transplantation* 60: 132–137, 1995
4. Glicklich D, Kapoian T, Mian H, Gilman J, Tellis V, Croizat H: Effects of erythropoietin, angiotensin II and angiotensin-convert-

ing enzyme inhibitor on erythroid precursors in patients with post transplant erythrocytosis. *Transplantation* 68: 62–66, 1999
5. Wolf G: Angiotensin as a renal growth promoting factor. *Adv Exp Med Biol* 377: 225–235, 1995
6. Chung O, Stoll M, Unger T: Physiologic and pharmacologic implications of AT_1 versus AT_2 receptors. *Blood Press* 2[Suppl]S47–S52:1996
7. Griendling KK, Ushio-Fukai M, Lassegue B, Alexander RW: Angiotensin II signaling in vascular smooth muscle. New concepts. *Hypertension* 29: 266–373, 1997
8. Mrug M, Stopka T, Julian BA, Prchal JF, Prchal JT: Angiotensin II stimulates proliferation of normal early erythroid progenitors. *J Clin Invest* 100: 2310–2314, 1997
9. Julian BA, Brantley RR, Barker CU, Stopka T, Gaston RS, Cortis JJ, Lee JY, Prchal JT: Losartan, an angiotensin II type 1 receptor antagonist, lowers hematocrit in post transplant erythrocytosis. *J Am Soc Nephrol* 9: 1104–1108, 1998
10. Perazella M, McPhedran P, Klinger A, Lorbes M, Levy E, Bia MJ: Enalapril treatment of post transplant erythrocytosis: Efficacy independent of circulating erythropoietin levels. *Am J Kidney Dis* 26: 495–500, 1995
11. Morrone LF, DiPeolo S, Logoluso F, Schena A, Stallone G, Giorgino F, Schena FP: Interference of angiotensin-converting enzyme inhibitors on erythropoiesis in kidney transplant recipients. *Transplantation* 64: 913–918, 1997
12. Brox AG, Mangel J, Hanley JA, St. Louis G, Mongrain S, Gagnon RF: Erythrocytosis after renal transplantation represents an abnormality of insulin-like growth factor-1 and its binding proteins. *Transplantation* 66: 1053–1058, 1998
13. Sharfi AM, Schiffrin EL: Apoptosis in vasculature of spontaneously hypertensive rats—effect of an angiotensin converting enzyme inhibitor and a calcium channel antagonist. *Am J Hypertens* 11: 1108–1116, 1998
14. Buemi M, Allegra A, Marino D, Marino MT, Medici MA, DePasquale G, Ruello A, Croica F, Frisina N: Does Captopril have a direct pro-apoptotic effect? *Nephron* 81: 99–101, 1999
15. Gomez RA, Norwood VF: Developmental consequences of the renin-angiotensin system. *Am J Kidney Dis* 26: 409–131, 1995
16. Koury MJ, Bondurant MC: The molecular mechanism of erythropoietin action. *Eur J Biochem* 210: 649–657, 1992
17. Boyer SM, Bishop TR, Rogers OC, Noyes AN, Frelin LP, Hobbs S: Roles of erythropoietin, insulin-like growth factor 1, and unidentified serum factors in promoting maturation of purified murine erythroid colony-forming units. *Blood* 80: 2503–2512, 1992
18. Croizat H, Billet HH, Nagel RL: Heterogeneity in the properties of burst-forming units of erythroid lineage in sickle cell anemia. *Blood* 75: 1006–1010, 1990
19. Reutelingsperger CP, Van Heerde WL: Annexin V, the regulator of phosphatidylserine-catalyzed inflammation and coagulation during apoptosis. *Cell Molec Life Sci* 53: 527–532, 1997
20. Feldenberg LR, Thevananther S, Del Rio M, De Leon M, Devarajan P: Partial ATP depletion induces Fas- and caspase-mediated apoptosis in MDCK cells. *Am J Physiol* 276 (*Renal Physiol* 45): F837–F846, 1999
21. Correa PN, Eskinazi D, Axelrad AA: Circulating erythroid progenitors in polycythemia vera are hypersensitive to insulin-like growth factor 1 in vitro: Studies in an improved serum-free medium. *Blood* 83: 99–112, 1994
22. Brox A, Congote LF, Faford J, Fauser AA: Identification and characterization of an 8-kd peptide stimulating erythropoiesis. *Exp Hematol* 17: 769–773, 1989
23. Congote LF, Brox A, Lin FK, Lu HS, Fauser AA: The N-terminal sequence of the major erythropoietic factor of an anephric patient is identical to insulin-like growth factor 1. *J Clin Endocrinol Metab* 72: 727–729, 1991
24. Brox AG, Zhang F, Guyda H, Gagnon RF: Subtherapeutic erythropoietin and insulin-like growth factor 1 correct the anemia of chronic renal failure in the mouse. *Kidney Int* 50: 937–943, 1996
25. Ratajczak J, Zhang Q, Pertusini E, Wojczyk BS, Wasik MA, Ratajczak MZ: The role of insulin and insulin-like growth factor 1 in regulating human erythropoiesis. Studies in vitro under serum-free conditions: Comparison to other cytokines and growth factors. *Leukemia* 12: 371–381, 1998
26. Shih LY, Huang JY, Lee CT: Insulin-like growth factor 1 plays a role in regulating erythropoiesis in patients with end-stage renal disease and erythrocytosis. *J Am Soc Nephrol* 10: 315–322, 1999
27. Urena P, Bonnardeaux A, Eckardt KU, Kurtz A, Drucke TB: Insulin-like growth factor 1—a modulator of erythropoiesis in uremic patients. *Nephrol Dial Transplant* 7: 40–44, 1992
28. Julian BA, Gaston RS, Barker CV, Krystal G, Diethelm AG, Curtis JJ: Erythropoiesis after withdrawal of enalapril in post transplant erythrocytosis kidney. *Kidney Int* 46: 1397–1403, 1994
29. Aeberhard JM, Schneider PA, Vallotton MB, Kurtz A, Leski M: Multiple site estimates of erythropoietin and renin in polycythemic kidney transplant patients. *Transplantation* 50: 613–616, 1990
30. Conlon PJ, Farrell J, Donahue J, Carmody M, Walsche JJ: The beneficial effect of enalapril on erythrocytosis after renal transplantation. *Transplantation* 56: 217–219, 1993
31. Gossmann J, Thurman P, Bachmann T, Weller S, Kachel HG, Schoeppe W, Scheuermann EH: Mechanism of angiotensin converting enzyme inhibitor-related anemia in renal transplant recipients. *Kidney Int* 50: 973–978, 1996
32. Kessler M, Hestin D, Mayeaux D, Mertes DM, Renoult E: Factors predisposing to post transplant erythrocytosis. *Clin Nephrol* 45: 83–89, 1996
33. Perazella M, McPhedran P, Kliger A, Lorber M, Levy E, Bia MJ: Enalapril treatment of post transplant erythrocytosis efficacy independent of circulating erythropoietin levels. *Am J Kidney Dis* 26: 495–500, 1995
34. Lee DBN: Cyclosporine and the renin-angiotensin axis. *Kidney Int* 52: 248–260, 1997
35. Ueda N, Kaushal GP, Shah SV: Apoptotic mechanisms in acute renal failure. *Am J Med* 108: 403–415, 2000
36. Papayannopoulou T, Abkowitz J: Biology of erythropoiesis, erythroid differentiation and maturation. In: *Hematology—Basic Principles and Practice*, edited by Hoffman R, Benz EJ, Stattil SJ, Fune B, Cohen HJ, Silberstein LE, New York, Churchill Livingstone, 1995, pp 242–254
37. Shan R, Price JO, Gaarde WA, Monia BP, Krantz SB, Zhao ZJ: Distinct roles of JNKs/P38 MAP Kinase and ERKs in apoptosis and survival of HCD-57 cells induced by withdrawal or addition of erythropoietin. *Blood* 94: 4067–4076, 1999