SEMINARS IN
NEPHROLOGY

Pathogenesis of Renal Anemia

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Anemia is a common complication of chronic kidney disease. Although mechanisms involved in the pathogenesis of renal anemia include chronic inflammation, iron deficiency, and shortened half-life of erythrocytes, the primary cause is deficiency of erythropoietin (EPO). Serum EPO levels in patients with chronic kidney disease are usually within the normal range and thus fail to show an appropriate increase with decreasing hemoglobin levels, as found in nonrenal anemias. Studies elucidating the regulation of EPO expression led to the identification of the hypoxia inducible factor–hypoxia responsive element system. However, despite much progress in understanding the molecular mechanisms through which cells can sense oxygen availability and translate this information into altered gene expression, the reason why EPO production is inappropriately low in diseased kidneys remains incompletely understood. Both alterations in the function of EPO-producing cells and perturbations of the oxygen-sensing mechanism in the kidney may contribute. As with other anemias, the consequences of renal anemia are a moderate decrease in tissue oxygen tensions and counterregulatory mechanisms that maintain total oxygen consumption, including a persistent increase in cardiac output.

KEYWORDS erythropoietin, anemia, hypoxia, inducible factor, chronic kidney disease, hypoxia

Adenosine triphosphate (ATP) serves as the primary energy currency of the cell. In most cells at least 90% of the molecular oxygen consumed is used for oxidative phosphorylation to produce ATP, and the remaining oxygen is used in a wide variety of specialized metabolic reactions. This is such an efficient system that the total ATP yield is 38 per mole of glucose oxidized, whereas anaerobic glycolysis produces only 2 ATP per mole of glucose.

In human beings at rest, about 250 mL of oxygen are consumed and 200 mL of carbon dioxide are produced per minute. During exercise, these quantities increase 10-fold. The red blood cells (erythrocytes) carry hemoglobin (Hb) in the circulation and play an essential role in oxygen delivery. Oxygen is relatively insoluble in water, and the development of Hb makes possible the transportation of a hundred times as much oxygen as could be carried by the plasma alone.

In 1893 the scientist Friedrich Miescher,[1] who later discovered DNA, reported a reproducible increase in his Hb concentration and red cell count when entering a sanatorium in the Alps. He realized that this increase occurred in response to reduced oxygen availability. Studies in the 1950s showed that there was a humoral factor stimulating erythropoiesis. Hypoxia-induced erythropoiesis in rats was diminished by bilateral nephrectomy, and this experiment clarified the role of the kidney in producing erythropoietic-stimulating factor, later called *erythropoietin* (EPO).[2]

EPO was purified in 1977 using a concentration of 2,550 L urine from patients with aplastic anemia.[3] The human EPO gene was cloned in 1983, and this enabled the expression of the mature glycoprotein hormone from Chinese hamster ovary cells and the mass production of recombinant human EPO (epoetin) to be available for clinical use.[4,5] The development of epoetin as a therapeutic agent is one of the major advances in the management of patients with renal failure over the past few decades. In fact, renal anemia nowadays can be considered as the consequence of chronic kidney disease (CKD), that is most accessible to therapeutic intervention. Nevertheless, the reasons for inappropriately low EPO production by diseased kidneys are still far from clear.

Epidemiology of Renal Anemia

In 1836, Bright[6] first described the association between anemia and chronic renal failure, and Brown and Roth[7] later

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concluded in 1922 that the anemia of chronic nephritis was caused by decreased bone marrow production. By 1933 Parsons and Ekola-Strolberg[8] recognized that the Hb concentration in uremia had roughly the same prognostic significance as the creatinine level. Before epoetin became available, about 25% of hemodialysis (HD) patients needed regular transfusions of red blood cells.

Anemia commonly contributes to poor quality of life in patients with CKD. Although the prevalence of anemia increases with diminishing renal function, a normochromic and normocytic anemia already can be observed at a relatively early stage of renal dysfunction. There is usually hypoplasia of the erythroid precursors in the bone marrow with normal leukopoiesis and megakaryocytopoiesis. Both the Third National Health and Nutrition Examination Survey III and the National Kidney Foundation: Kidney Early Evaluation Program showed that the risk of anemia significantly increases when the glomerular filtration rate decreases to less than 60 mL/min/1.73 m^2.[9,10] Although 5% of the US population with a glomerular filtration rate between 30 and 59 were anemic, 44% of patients with a glomerular filtration rate between 15 and 29 mL/min per 1.73 m^2 showed a decrease in Hb level.[9] Analysis of data from the Invecchiare in Chianti (InCHIANTI) study, a population-based study performed in a sample of community-dwelling older persons living in Italy, also revealed that both men and women with a creatinine clearance of 60 mL/min or less were significantly more likely to have anemia compared with individuals with a creatinine clearance of more than 90 mL/min.[11] However, when the confounding effect of age on the relationship between kidney function and anemia was removed from the analysis, only participants with a creatinine clearance of 30 mL/min or less showed a higher prevalence of anemia in this population.

Production of EPO in the Kidney

The gene encoding human EPO is located on chromosome 7[12] and encompasses about 3,000 base pairs. It contains 5 exons and 4 introns and encodes for a 193–amino-acid polypeptide.[4,5] The recent cloning of the EPO gene from the puffer fish showed that the synteny of genes at the EPO locus is highly conserved during evolution.[13]

A 27–amino-acid leader sequence at the N-terminal part and a carboxy-terminal arginine molecule of human EPO are cleaved off during secretion so that the mature 34-kd glycoprotein contains 165 amino acids.[14] Roughly 30% to 40% of the molecular mass is made up of carbohydrate chains, which are important to prevent degradation and delay clearance of EPO from the circulation and therefore are necessary for in vivo activity. Epoetin used clinically is therefore highly glycosylated, although its carbohydrate chains are not identical to that of natural EPO. Darbepoetin, a pharmaceutically engineered EPO, has a significantly longer half-life because it contains more glycosylation sites on the polypeptide backbone and has more carbohydrate chains than natural EPO.

The body contains no significant stores of EPO, and any change in the serum EPO concentration results from a change in the rate of production. Circulating EPO in the normal adult is produced mainly by the peritubular fibroblast-like interstitial cells of the kidney.[15–17] When the hematocrit level is in the normal range, the kidney produces a low level of EPO, with EPO expression limited to a small number of these fibroblasts in the deep cortex and superficial outer medulla. The increased production of EPO under anemic circumstances involves progressive recruitment of additional interstitial fibroblasts in a pattern that spreads outward from the deep cortex toward the capsule. Even at maximal stimulation, less than 20% of cortical fibroblast-like cells in the cortical labyrinth express EPO and thus it is possible that only a yet-unidentified subgroup of specialized interstitial cells produces the hormone. The explanation of why the mammalian kidney, a nonerythropoietic organ, plays the important role of producing EPO is not immediately obvious. One clue may lie in the evolutionary history, since erythropoietic tissue is found only in the kidney of most teleosts, chondrosteans, and holosteans.[18] An alternative explanation may be that changes in renal blood flow influence both oxygen delivery and—via alterations in filtered sodium load—oxygen consumption, which renders renal partial pressure of oxygen (P_{O_2}) to some extent independent of changes in blood flow.

The relative contribution of the liver to erythropoiesis is primarily age dependent. The liver is the predominant production site during fetal, and in some species also early, postnatal life.[19] The liver does not normally compensate for failure of the renal production of EPO in adults.

EPO Receptor and Signaling

EPO is considered an essential growth factor for late erythroid progenitor cells. This view has been confirmed by observations in patients developing anti-EPO antibodies in response to epoetin.[20] The occurrence of such antibodies leads to an almost complete cessation of red cell production, with an absence of erythroid progenitors from the bone marrow, very low reticulocyte counts, and regular transfusion dependence.

Physiologically, activation of the EPO receptor on the immature erythroid cells by EPO generates an intracellular signal that promotes the survival of these cells, which otherwise would undergo apoptosis. The EPO receptor is a member of the cytokine-receptor superfamily. Dimerization of the receptor results in autophosphorylation and activation of several kinases that initiate multiple signal transduction pathways. The phosphorylation of signal transducer and activator of transcription 5 (STAT5) leads to its homodimerization, which then allows the receptor to enter the nucleus and enhance the transcription of various genes, including antiapoptotic Bcl-X_L.[21] The phosphorylation of phosphatidylinositol 3-kinase activates its kinase activity, which in turn phosphorylates protein kinase B (Akt).[22] Akt then will induce cytoplasmic retention of forkhead box O transcription factors through their phosphorylation, and therefore inhibit proapoptotic molecules, such as Fas ligand or Bim.[23] Akt also will phosphorylate and inactivate other proapoptotic molecules, such as glycogen synthase kinase-3β, caspase 9, or Bad. Moreover, inhibitor of nuclear factor-κB (NF-κB) also is

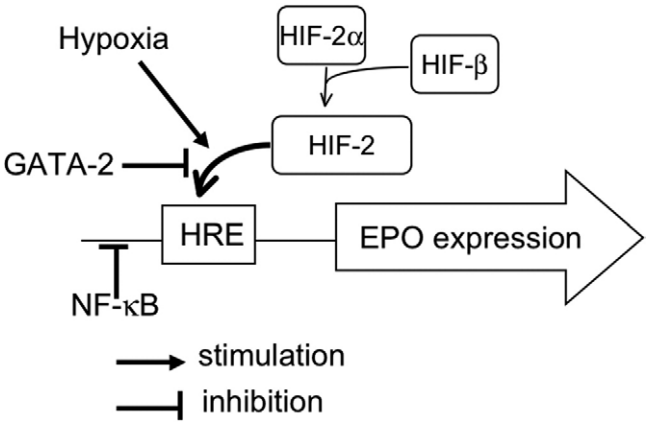

Figure 1 Regulation of EPO expression.

phosphorylated by Akt, which allows the release of the transcription factor NF-κB. Translocation of NF-κB into the nucleus results in transcription of many target genes including antiapoptotic molecules, such as X-linked inhibitor of apoptosis and cellular inhibitor of apoptosis protein 2 (c-IAP2). EPO also promotes cell proliferation by activation of the Ras/mitogen-activated protein kinase pathway.[24]

Regulation of EPO Expression by Hypoxia-Inducible Transcription Factors

The main determinant of EPO synthesis is the transcriptional activity of its gene, which is related to local oxygen tensions. EPO production is related inversely to oxygen availability, so that an effective feedback loop is established, which controls erythropoiesis.[25] In isolated perfused kidneys, EPO messenger RNA and EPO secretion are modulated in response to alterations of the oxygen tension of the perfusate.[26,27] Thus, although humoral signals from extrarenal sensing systems may contribute to the renal control of EPO production under certain conditions, all the components necessary for the detection of hypoxia and production of EPO are equipped within the kidney.

The oxygen-dependent control of EPO production is achieved by hypoxia-inducible transcription factors (HIFs), consisting of a constitutive β-subunit and 1 of 2 alternative oxygen-regulated HIF α subunits (HIF-1α and HIF-2α)[28,29] (Fig 1). In the presence of oxygen (normoxia) the HIF α subunits are hydroxylated, which targets them for proteasomal degradation. Under hypoxia, because of the lack of molecular oxygen, HIF cannot be hydroxylated and thereby is stabilized. Accumulated HIF binds to the key sequence among several regulatory DNA sequences in the neighborhood of the EPO gene, the hypoxia responsive element to the HRE. It is composed of the nucleotides [A/G]CGTG. Binding of HIF to HRE activates the transcription of EPO.

Although most cell types express HIF-1α and HIF-1β, HIF-2α (also called EPAS: Endothelial Per-ARNT-Sim domain protein, or HLF: HLF-1α-like factor) shows a more restricted pattern of expression.[30] Whether HIF-1α and HIF-2α have distinct roles is an interesting and important subject for investigation. By using microarray analysis, Hu et al[31] showed unique regulation of some glycolytic genes by 1 HIF isoform. Several endothelial cell–specific genes have been shown to be regulated exclusively by HIF-2α.[32] Although HIF-1α was the first transcription factor identified through its ability to bind to an enhancer sequence of the EPO gene, more recent evidence suggests that HIF-2α is responsible for the regulation of EPO. Analysis of HIF-2α knockdown mice revealed that EPO gene expression was affected significantly, in parallel with HIF-2α expression.[33] A predominant role of HIF-2α in the regulation of EPO expression in cell lines also was shown in a study using RNA interference to determine the contribution of HIF-1α versus HIF-2α to the hypoxic gene induction.[34] Although most genes tested were responsive only to the HIF-1α short interference (si)RNA, EPO showed responsiveness only to HIF-2α knockdown.

In addition, in rat kidneys in vivo, HIF-1α and HIF-2α were detected in different cell populations.[35] Although HIF-1α was expressed in epithelial cells, HIF-2α was found in peritubular fibroblasts and endothelial cells. The detection of HIF-2α in the EPO-producing renal fibroblasts further supported a regulatory role of HIF-2α in the regulation of EPO expression.

Other Transcriptional Regulators of EPO

Although HIF-1α and HIF-2α can activate the EPO gene, GATA-2 and NF-κB inhibit EPO gene transcription.

Imagawa et al[36,37] showed that GATA-2 inhibits EPO gene transcription by binding to the EPO promoter under normoxic conditions. For example, the nitric oxide synthase inhibitor N(G)-monomethyl-L-arginine decreases EPO production by increasing GATA-2 DNA binding.[38] In contrast, the addition of L-arginine inhibited the binding activity of GATA-2 and rescued decreased expression of EPO.[39] Furthermore, the EPO promoter and the 5′ flanking region contain binding sites for NF-κB.[40] Evidence suggests that both GATA-2 and NF-κB are involved in the inhibition of EPO gene expression under inflammatory conditions.[41] The administration of a GATA-specific inhibitor also ameliorated anemia induced by a variety of inflammatory cytokines in mice.[42] Because uremia is a proinflammatory status, it is possible that these inhibitory transcriptional factors also contribute to the regulation of EPO expression in vivo.

EPO Deficiency and Renal Anemia

Normal serum EPO concentrations in human beings are of the order of 10 to 30 mU/mL as determined by radioimmunoassays, which corresponds to between 2 and 7 pmol/L. EPO concentrations are increased under a variety of conditions, largely reflecting alterations of oxygen delivery to tis-

Table 1 Pathogenic Factors of Renal Anemia

EPO deficiency
Hemolysis
Absolute iron deficiency
Functional iron deficiency
Folic acid deficiency
Carnitine deficiency
Chronic inflammation
Aluminium intoxication
Hyperparathyroidism with myelofibrosis
External blood loss
Bone-marrow suppression induced by retained toxic metabolites
Drugs

sues. The expected compensatory response to anemia is a heightened rate of erythropoiesis, with an inverse relationship between the concentration of the hormone and the Hb concentration.[43] In severely anemic patients, up to 1,000-fold increases in EPO levels can be found.

Although the anemia of chronic renal failure is a complex disorder in which many factors may play a role (Table 1), the main defect is absolute or relative EPO deficiency. In most patients with substantially impaired renal function, EPO production is impaired at any given hematocrit concentration.[44] Values for serum EPO levels in patients with renal disease may vary with the assay method used, but an important point is that even the occasional increased EPO levels actually are decreased in relation to the degree of anemia. Fehr et al[45] studied 395 patients randomly chosen from more than 5,000 consecutive patients investigated by coronary angiography at a single center. Although Hb negatively regulated EPO in patients with a creatinine clearance of more than 40ml/min, EPO levels remained stable below this cut-off level. Serum EPO concentrations are also very low in anephric patients, and EPO probably is produced by the liver in this situation.[46]

Why EPO production remains inappropriately low in patients with kidney disease is unclear. In principle, this could be because the EPO-producing cells are lost, because they experience higher levels of oxygen for a given hematocrit level, or because the relationship between local oxygenation and EPO production is altered.[28] Several lines of evidence suggest that diseased kidneys are hypoxic and that this chronic decrease in oxygen availability in fact may contribute to disease progression.[47,48] This could indicate that a defect in EPO production rather than a lack of the hypoxic stimulus leads to the failure of EPO production. On the other hand, preliminary evidence suggests that treatment with a pharmacologic inhibitor of HIF degradation can stimulate EPO production in CKD patients,[49] which clearly suggests that the production capacity for the hormone is maintained.

Impaired EPO Production in Diabetic Nephropathy

Although renal anemia develops largely independent of the underlying cause of kidney disease, anemia appears to develop earlier and to be more severe in patients with diabetes.[50,51] Bosman et al[52] compared 27 type 1 diabetic patients with nephropathy and 26 nondiabetic patients with glomerulonephritis and persistent proteinuria. Although one half of the diabetic nephropathy patients were anemic, none of the glomerulonephritis patients showed a decrease in Hb levels. In the diabetic nephropathy group, serum EPO concentrations failed to increase in response to anemia compared with the response seen in patients with microcytic anemia, indicating that the anemia of the diabetic nephropathy group was associated with EPO deficiency. Craig et al[53] studied 62 patients with type 2 diabetes for a median follow-up period of 7 years. Although only 16% of subjects in the group were overtly anemic, all subjects had an ongoing small but significant decrease in Hb level since presentation. Furthermore, subjects in this study lacked the expected reticulocyte response. El-Achkar et al[54] used National Kidney Foundation Kidney Early Evaluation Program 2.0 screening data to determine the prevalence of anemia by level of kidney function and diabetes status. Their analysis of 5,380 participants showed that anemia prevalence in stages II to V of kidney disease [ie, with descending estimated glomerular filtration rate (e GFR)] were 8.7%, 7.5%, 22.2%, and 52.4%, respectively, in participants with diabetes, compared with 6.9%, 5.0%, 7.9%, and 50.0%, respectively, in persons without diabetes. In a multivariable model, those with diabetes had significantly increased odds of anemia.

The precise mechanisms of the early development of anemia in diabetic patients are unknown. Thomas et al[55] obtained clinical data on 604 patients with type 2 diabetes in a single clinic at Austin Health in Melbourne, Australia and found that even in the absence of renal impairment, 71% of anemic patients had functional EPO deficiency. Their findings confirmed the failure of the kidney to produce EPO in response to a decreasing Hb level as a key component to anemia in diabetes. In addition to EPO deficiency caused by tubulointerstitial changes, which is an early morphologic alteration of diabetic nephropathy, increased serum advanced glycation end-products may participate in the pathogenesis of anemia in diabetic kidney disease.[56] Recent studies by Katevetin et al[57] showed that high glucose levels blunted activation of the HIF-HRE pathway in cultured tubular cells. Restoration of the HIF-HRE activity by antioxidant α-tocopherol indicated that inhibition of the HIF-HRE pathway by high glucose levels is mediated by oxidative stress. Although autonomic neuropathy also may be involved,[58] observations in patients with renal transplants[59,60] and experimental data[61] indicate that no essential control of EPO production occurs via the renal nerves.

Increased Destruction of Red Blood Cells in Uremia

Although insufficient production of EPO by the diseased kidneys is the primary cause of renal anemia, additional factors contribute to renal anemia.

Hemolysis in the terminal stages of kidney disease was

observed by several investigators,[62,63] and Eschbach et al[64] observed a diminished red blood cell lifespan in HD patients using P-labeled di-isopropyl fluorophosphate. A correlation between red blood cell survival time and serum blood urea nitrogen level was inverse,[65] and exposure to uremic serum shortens the survival of erythrocytes from healthy subjects.[66,67] These various studies have estimated that red blood cell survival may be as low as half of normal levels.

The primary cause of hemolysis in renal failure may be the retention of uremic solutes in plasma. The inhibitory effects of uremic plasma on the activity of membrane calcium ATPase[68] have been suggested as contributing to hemolysis. Alterations in the structure and function of erythrocyte plasma membrane including reduced membrane fluidity and impairment of metabolic parameters also may shorten the erythrocyte lifespan in uremia.[69] Oxidative stress associated with kidney failure also may contribute to uremic anemia by shortening erythrocyte survival.

It should be noted, however, that normally increased bone marrow production could easily offset the mild to moderate hemolysis that occurs in uremia. Only in the presence of depressed erythropoiesis does the reduced red blood cell lifespan become an important contributor to the development of renal anemia.

In addition to destruction of erythrocytes, uremia contributes to renal anemia via anorexia, leading to a reduced intake of hemoglobin substrates such as vitamins and iron.

Uremic Suppression of Erythropoiesis

The role of uremic suppression on erythropoiesis remains controversial.[70] The presence of inhibitors of erythropoiesis in uremic plasma was postulated in the light of the report that anemia improves after HD is started.[71] It was shown later that the hematocrit level increases after the start of regular dialysis despite a significant decrease in endogenous serum EPO levels, suggesting that HD removes a bone marrow inhibitor.[72,73] Adequacy of dialysis is a key to correcting anemia and optimizing the use of recombinant human EPO (rHuEPO) in a number of HD patients.[74]

Despite these reports, sheep and human erythroid progenitor cells, regardless of whether they were from a uremic or nonuremic environment, were not suppressed by autologous uremic sera.[75] Furthermore, assessment of the erythropoietic response to EPO showed that the acute response to EPO was similar in 22 normal subjects and 24 HD patients.[76] These results suggest that chronic uremia does not alter the responsiveness to EPO in vivo.

Iron Deficiency

Iron is a critical body substance, transporting oxygen to tissues via Hb and functioning as a cofactor in a number of enzyme systems. The most common factor that confounds renal anemia is iron deficiency, whether it is related to or independent of blood loss from repeated laboratory testing, needle punctures, or blood retention in the dialyzer and tubing at the end of each dialysis treatment.[77] It has been estimated that 1 to 3 g of iron are lost annually from these causes,[78] and uptake of iron by intestinal mucosal cells and iron retention also may be impaired in dialysis patients.

Deficient available iron is the most common cause of initial poor response to epoetin, as well as acquired refractoriness to this agent. A recent multicenter trial of peritoneal dialysis patients also showed that iron-treated patients showed a calculated net EPO dose decrease compared with untreated control subjects.[79] The administration of iron to 47 patients with CKD resulted in an increase of Hb level, and 26 patients reached the target Hb level of 12 g/dL even without EPO.[80]

Chronic Inflammation

Uremia is a chronic inflammatory state,[81,82] and thus patients with renal failure may develop anemia and become refractory to EPO because of mechanisms associated with chronic inflammation.[83] A significant association has been shown between hyporesponsiveness to EPO and high levels of inflammatory markers in HD patients.[84–86] Hyporesponsiveness to EPO in patients with chronic inflammation often can be explained by functional iron deficiency. This is characterized by apparently insufficient available iron to keep up with the demands of erythropoiesis.

The mechanisms of functional iron deficiency may involve increased levels of circulating cytokines that are capable of inducing macrophages of the reticuloendothelial system to more avidly take up and hold on to iron. Cytokines also may decrease endogenous EPO production as described earlier, or decrease the responsiveness of erythroid precursor cells to endogenous or exogenous EPO. In particular, interleukin-1 β and tumor necrosis factor- α have been shown to have both of these effects.[87,88]

Recent studies have indicated a potential contribution of hepcidin in dysregulation of iron metabolism in patients with kidney disease.[89,90] Hepcidin evolves as a potent regulator of the body's iron distribution, piloting the flow of iron via, and directly binding to, the cellular iron exporter ferroportin.[89] Hepcidin is expressed in the liver, distributed in blood, and excreted in urine. The hepcidin-ferroportin axis dominates the iron egress from all cellular compartments that are critical to iron homeostasis. The gene that encodes hepcidin expression is subject to regulation by proinflammatory cytokines, such as interleukin-6 and interleukin-1, and excessive hepcidin production contributes to the functional iron deficiency and associated anemia during inflammatory states. A semiquantitative assay for hepcidin using surface-enhanced laser desorption ionization time of flight mass spectrometry revealed accumulation of hepcidin in the serum of HD patients.[90] The level of serum hepcidin correlated well with the levels of serum ferritin and serum interleukin-6. Thus, accumulation of hepcidin may contribute to the pathogenesis of renal anemia by decreasing the available iron for hematopoiesis.

Other Contributing Factors

In patients with renal disease, agents that block the renin-angiotensin system also may contribute to reduced EPO levels and anemia.[91] Sophisticated experiments by Kato et al[92] showed a molecular interaction of erythropoiesis and the renin-angiotensin system in transgenic mice. Although animals carrying both the human renin and human angiotensinogen genes displayed persistent erythrocytosis, the introduction of both transgenes into the AT1a receptor null background restored the hematocrit level in the compound mice to the normal level. Plasma EPO levels and kidney EPO messenger RNA expression in the double transgenic mice were increased significantly compared with those of the wild-type control, whereas the increased plasma EPO levels were attenuated significantly in the compound mice. These results provide clear genetic evidence that the activated renin-angiotensin system enhances erythropoiesis through the AT1a receptor and that this effect is at least in part mediated by the increase of plasma EPO levels. In the Reduction of Endpoints in non-insulin dependent diabetes mellitus (NIDDM) with the Angiotensin II Antagonist Losartan (RENAAL) study in patients with overt nephropathy, Hb levels were a mean of 0.4 g/dL lower in patients treated with the angiotensin receptor antagonist, losartan, than in those receiving placebo.[93]

Other factors that may contribute to the development of renal anemia include severe hyperparathyroidism,[94] aluminum overload, carnitine deficiency, and myelosuppressive agents, particularly in transplant recipients.

Consequences of Renal Anemia

Anemia in CKD patients is associated inversely with quality of life and life expectancy and associated directly with cardiovascular morbidity and progressive loss of renal function. To which extent these relationships reflect causality so that anemia correction improves patient well-being and outcome is a matter of ongoing investigation and debate.

From a pathophysiologic point of view it is clear that unless anemia is very severe (Hb level <4 g/dL) whole-body oxygen consumption is not reduced, despite the reduction in oxygen-carrying capacity. This mainly is because of 2 compensatory mechanisms: first, an increase in oxygen extraction and, second, an increase in cardiac output. The former inevitably will reduce tissue oxygen tensions. In fact, the production of EPO in the normal kidney shows how sensitive cells and tissues can be to such changes in oxygen tensions, even if the Hb concentration is reduced only very mildly. The second mechanism (ie, a chronic increase in cardiac output) is considered as one of the possible mechanisms through which anemia may promote cardiac workload and left ventricular hypertrophy.

References

1. Miescher F: Ueber die Beziehungen zwischen Meereshoehe und Beschaffenheit des Blutes. Korrespbl Schweiz Arz 23:809-830, 1893
2. Jacobson LO, Goldwasser E, Fried W, et al: Role of the kidney in erythropoiesis. Nature 179:633-634, 1957
3. Miyake T, Kung CK, Goldwasser E: Purification of human erythropoietin. J Biol Chem 252:5558-5564, 1977
4. Lin FK, Suggs S, Lin CH, et al: Cloning and expression of the human erythropoietin gene. Proc Natl Acad Sci U S A 82:7580-7584, 1985
5. Jacobs K, Shoemaker C, Rudersdorf R, et al: Isolation and characterization of genomic and cDNA clones of human erythropoietin. Nature 313:806-810, 1985
6. Bright R: Cases and observations illustrative of renal disease accompanied with the secretion of albuminous urine. Guy's Hosp Rep 1:338-400, 1836
7. Brown GE, Roth GM: The anemia of chronic nephritis. Arch Intern Med 30:817-840, 1922
8. Parsons L, Ekola-Strolberg M: Anemia in azotemia. Am J Med Sci 185: 181-190, 1933
9. Astor BC, Muntner P, Levin A, et al: Association of kidney function with anemia: The Third National Health and Nutrition Examination Survey (1988-1994). Arch Intern Med 162:1401-1408, 2002
10. NKF Kidney Early Evaluation Program: Anemia & chronic kidney disease. Am J Kidney Dis 45:S71-S80, 2005 (suppl 2)
11. Ble A, Fink JC, Woodman RC, et al: Renal function, erythropoietin, and anemia of older persons: The InCHIANTI study. Arch Intern Med 165: 2222-2227, 2005
12. Law ML, Cai GY, Lin FK, et al: Chromosomal assignment of the human erythropoietin gene and its DNA polymorphism. Proc Natl Acad Sci U S A 83:6920-6924, 1986
13. Chou C-F, Tohari S, Brenner S, et al: Erythropoietin gene from a teleost fish, Fugu rubripes. Blood 104:1498-1503, 2004
14. Recny MA, Scoble HA, Kim Y: Structural characterization of natural human urinary and recombinant DNA-derived erythropoietin. Identification of des-arginine 166 erythropoietin. J Biol Chem 262:17156-17163, 1987
15. Bachmann S, Le Hir M, Eckardt KU: Co-localization of erythropoietin mRNA and ecto-5′-nucleotidase immunoreactivity in peritubular cells of rat renal cortex indicates that fibroblasts produce erythropoietin. J Histochem Cytochem 41:335-341, 1993
16. Maxwell PH, Osmond MK, Pugh CW, et al: Identification of the renal erythropoietin-producing cells using transgenic mice. Kidney Int 44: 1149-1162, 1993
17. Koury ST, Koury MJ, Bondurant MC, et al: Quantitation of erythropoietin-producing cells in kidneys of mice by in situ hybridization: Correlation with hematocrit, renal erythropoietin mRNA and serum erythropoietin concentration. Blood 74:645-651, 1989
18. Wickramasinghe SN: Erythropoietin and the human kidney: Evidence for an evolutionary link from studies of salmo gairdneri. Comp Biochem Physiol 104A:63-65, 1993
19. Eckardt KU, Ratcliffe PJ, Tan CC, et al: Age-dependent expression of the erythropoietin gene in rat liver and kidneys. J Clin Invest 89:753-760, 1992
20. Rossert J, Casadevall N, Eckardt KU: Anti-erythropoietin antibodies and pure red cell aplasia. J Am Soc Nephrol 15:398-406, 2005
21. Silva M, Benito A, Sanz C, et al: Erythropoietin can induce the expression of bcl-x(L) through Stat5 in erythropoietin-dependent progenitor cell lines. J Biol Chem 274:22165-22169, 1999
22. Damen JE, Mui AL, Puil L, et al: Phosphatidylinositol 3-kinase associates, via its Src homology 2 domains, with the activated erythropoietin receptor. Blood 81:3204-3210, 1993
23. Kashii Y, Uchida M, Kirito K, et al: A member of Forkhead family transcription factor, FKHRL1, is one of the downstream molecules of phosphatidylinositol 3-kinase-Akt activation pathway in erythropoietin signal transduction. Blood 96:941-949, 2000
24. Miura Y, Miura O, Ihle JN, et al: Activation of the mitogen-activated protein kinase pathway by the erythropoietin receptor. J Biol Chem 269:29962-29969, 1994
25. Eckardt KU, Kurtz A: Regulation of erythropoietin production. Eur J Clin Invest 35:13-19, 2005 (suppl 3)
26. Ratcliffe PJ, Jones RW, Phillips RE, et al: Oxygen-dependent modulation of erythropoietin mRNA levels in isolated rat kidneys studied by RNase protection. J Exp Med 172:657-660, 1990
27. Scholz H, Schurek HJ, Eckardt KU, et al: Oxygen-dependent erythro-

poietin production by the isolated perfused rat kidney. Pflugers Arch 418:228-233, 1991
28. Maxwell P: HIF-1: An oxygen response system with special relevance to the kidney. J Am Soc Nephrol 14:2712-2722, 2003
29. Ratcliffe PF, Schofield C: Oxygen sensing by HIF hydroxylases. Nat Rev Mol Cell Biol 5:343-354, 2004
30. Wiesener MS, Jurgensen JS, Rosenberger C, et al: Widespread hypoxia-inducible expression of HIF-2alpha in distinct cell populations of different organs. FASEB J 17:271-273, 2003
31. Hu CJ, Wang LY, Chodosh LA, et al: Differential roles of hypoxia-inducible factor 1alpha (HIF-1alpha) and HIF-2alpha in hypoxic gene regulation. Mol Cell Biol 23:9361-9374, 2003
32. Elvert G, Kappel A, Heidenreich R, et al: Cooperative interaction of hypoxia-inducible factor-2alpha (HIF-2alpha) and Ets-1 in the transcriptional activation of vascular endothelial growth factor receptor-2 (Flk-1). J Biol Chem 278:7520-7530, 2003
33. Morita M, Ohneda O, Yamashita T, et al: HLF/HIF-2alpha is a key factor in retinopathy of prematurity in association with erythropoietin. EMBO J 22:1134-1146, 2003
34. Warnecke C, Griethe W, Weidemann A, et al: Activation of the hypoxia-inducible factor-pathway and stimulation of angiogenesis by application of prolyl hydroxylase inhibitors. FASEB J 17:1186-1188, 2003
35. Rosenberger C, Mandriota S, Jurgensen JS, et al: Expression of hypoxia-inducible factor-1 and -2 in hypoxic and ischemic rat kidneys. J Am Soc Nephrol 13:1721-1732, 2002
36. Imagawa S, Izumi T, Miura Y: Positive and negative regulation of the erythropoietin gene. J Biol Chem 269:9038-9044, 1994
37. Imagawa S, Yamamoto M, Miura Y: Negative regulation of the erythropoietin gene expression by the GATA transcription factors. Blood 89: 1430-1439, 1997
38. Tarumoto T, Imagawa S, Ohmine K, et al: N(G)-monomethyl-L-arginine inhibits erythropoietin gene expression by stimulating GATA-2. Blood 96:1716-1722, 2000
39. Imagawa S, Tarumoto T, Suzuki N, et al: L-arginine rescues decreased erythropoietin gene expression by stimulating GATA-2 with L-NMMA. Kidney Int 61:396-404, 2002
40. Lee-Huang S, Lin JJ, Kung HF, et al: The human erythropoietin-encoding gene contains a CAAT box, TATA boxes and other transcriptional regulatory elements in its 5′ flanking region. Gene 128:227-236, 1993
41. La Ferla K, Reimann C, Jelkmann W, et al: Inhibition of erythropoietin gene expression signaling involves the transcription factors GATA-2 and NF-kappaB. FASEB J 16:1811-1813, 2002
42. Imagawa S, Nakano Y, Obara N, et al: A GATA-specific inhibitor (K-7174). FASEB J 17:1742-1744, 2003
43. Caro J, Brown S, Miller O, et al: Erythropoietin levels in uremic nephric and anephric patients. J Lab Clin Med 93:449-458, 1979
44. Eschbach JW: The anemia of chronic renal failure: Pathophysiology and the effects of recombinant erythropoietin. Kidney Int 35:134-148, 1989
45. Fehr T, Ammann P, Garzoni D, et al: Interpretation of erythropoietin levels in patients with various degrees of renal insufficiency and anemia. Kidney Int 66:1206-1211, 2004
46. Naets JP, Garcia JF, Tousaaint C, et al: Radioimmunoassay of erythropoietin in chronic uraemia or anephric patients. Scand J Haematol 37:390-394, 1986
47. Eckardt KU, Rosenberger C, Jurgensen JS, et al: Role of hypoxia in the pathogenesis of renal disease. Blood Purif 21:253-257, 2003
48. Nangaku M: Chronic hypoxia and tubulointerstitial injury: A final common pathway to end-stage renal failure. J Am Soc Nephrol 17:17-25, 2006
49. Urquilla P, Fong A, Oksanen S, et al: Upregulation of endogenous EPO in healthy subjects by inhibition of HIF-PH. J Am Soc Nephrol 15: 546A, 2004
50. Thomas MC, MacIsaac RJ, Tsalamandris C, et al: Unrecognized anemia in patients with diabetes: A cross-sectional survey. Diabetes Care 26: 1164-1169, 2003
51. Thomas MC, MacIsaac RJ, Tsalamandris C, et al: Anemia in patients with type 1 diabetes. J Clin Endocrinol Metab 89:4359-4363, 2004
52. Bosman DR, Winkler AS, Marsden JT, et al: Anemia with erythropoietin deficiency occurs early in diabetic nephropathy. Diabetes Care 24:495-499, 2001
53. Craig KJ, Williams JD, Riley SG, et al: Anemia and diabetes in the absence of nephropathy. Diabetes Care 28:1118-1123, 2005
54. El-Achkar TM, Ohmit SE, McCullough PA, et al: Higher prevalence of anemia with diabetes mellitus in moderate kidney insufficiency: The Kidney Early Evaluation Program. Kidney Int 64:1483-1488, 2005
55. Thomas MC, Tsalamandris C, MacIsaac R, et al: Functional erythropoietin deficiency in patients with type 2 diabetes and anaemia. Diabet Med 23:502-509, 2006
56. Thomas MC, Tsalamandris C, MacIsaac R, et al: Low-molecular-weight AGEs are associated with GFR and anemia in patients with type 2 diabetes. Kidney Int 66:1167-1172, 2004
57. Katevetin P, Miyata T, Inagi R, et al: High glucose blunts VEGF response to hypoxia via the oxidative stress-regulated HIF-HRE pathway. J Am Soc Nephrol 17:1405-1413, 2006
58. Bosman DR, Osborne CA, Marsden JT, et al: Erythropoietin response to hypoxia in patients with diabetic autonomic neuropathy and non-diabetic chronic renal failure. Diabet Med 19:65-69, 2002
59. Jeffrey RF, Kendall RG, Prabhu P, et al: Re-establishment of erythropoietin responsiveness in end-stage renal failure following renal transplantation. Clin Nephrol 44:241-247, 1995
60. Rejman AS, Grimes AJ, Cotes PM, et al: Correction of anaemia following renal transplantation: Serial changes in serum immunoreactive erythropoietin, absolute reticulocyte count and red-cell creatine levels. Br J Haematol 61:421-431, 1985
61. Eckardt KU, LeHir M, Tan CC, et al: Renal innervation plays no role in oxygen-dependent control of erythropoietin mRNA levels. Am J Physiol 263:F925-F930, 1992
62. Chaplin H Jr, Mollison PL: Red cell life-span in nephritis and in hepatic cirrhosis. Clin Sci 12:351-360, 1953
63. Joske RA, McAlister JM, Prankerd TA: Isotope investigations of red cell production and destruction in chronic renal disease. Clin Sci 15:511-522, 1956
64. Eschbach JW Jr, Funk D, Adamson J, et al: Erythropoiesis in patients with renal failure undergoing chronic dialysis. N Engl J Med 276:653-658, 1967
65. Shaw AB: Haemolysis in chronic renal failure. BMJ 2:213-216, 1967
66. Loge JP, Lange RD, Moore CV: Characterization of the anemia associated with chronic renal insufficiency. Am J Med 24:4-18, 1958
67. Kaye M: The anemia associated with renal disease. J Lab Clin Med 52:83-100, 1957
68. Lindner A, Gagne E-R, Zingraff J, et al: A circulating inhibitor of the RBC membrane calcium pump in chronic renal failure. Kidney Int 42:1328-1335, 1992
69. McGrath LT, Douglas AF, McClean E, et al: Oxidative stress and erythrocyte membrane fluidity in patients undergoing regular dialysis. Clin Chim Acta 235:179-188, 1995
70. Bonomini M, Sirolli V: Uremic toxicity and anemia. J Nephrol 16:21-28, 2003
71. Eschbach JW, Adamson JW, Cook JD: Disorders of red blood cell production in uremia. Arch Intern Med 126:812-815, 1970
72. Radtke HW, Frei U, Erbes PM, et al: Improving anemia by hemodialysis: Effect on serum erythropoietin. Kidney Int 17:382-387, 1980
73. Radtke HW, Klaussner A, Erbes PM, et al: Serum erythropoietin concentration in CRF: Relationship to degree of anemia and excretory renal function. Blood 54:877-884, 1979
74. Locatelli F: Optimizing dose and mode of renal replacement therapy in anaemia management. Nephrol Dial Transplant 17:S60-S65, 2002 (suppl 5)
75. Segal GM, Eschbach JW, Egrie JC, et al: The anemia of end-stage renal disease: Hematopoietic progenitor cell response. Kidney Int 33:983-988, 1988
76. Eschbach JW, Haley NR, Egrie JC, et al: A comparison of the responses to recombinant human erythropoietin in normal and uremic subjects. Kidney Int 42:407-416, 1992
77. Nissenson AR, Strobos J: Iron deficiency in patients with renal failure. Kidney Int 55:S18-S21, 1999

78. Akmal M, Sawelson S, Karubian F, et al: The prevalence and significance of occult blood loss in patients with predialysis advanced chronic renal failure, or receiving dialytic therapy. Clin Nephrol 42:198-202, 1994
79. Singh H, Reed J, Noble S, et al: Effect of intravenous iron sucrose in peritoneal dialysis patients who receive erythropoiesis-stimulating agents for anemia: A randomized, controlled trial. Clin J Am Soc Nephrol 1:475-482, 2006
80. Gotloib L, Silverberg DS, Fudin R, et al: Iron deficiency is a common cause of anemia in chronic kidney disease and can often be corrected with intravenous iron. J Nephrol 19:161-167, 2006
81. Kalantar-Zadeh K, Ikizler TA, Block G, et al: Malnutrition-inflammation complex syndrome in dialysis patients: Causes and consequences. Am J Kidney Dis 42:864-881, 2003
82. Stenvinkel P, Ketteler M, Johnson RJ, et al: IL-10, IL-6, and TNF-alpha: Central factors in the altered cytokine network of uremia—the good, the bad, and the ugly. Kidney Int 67:1216-1233, 2005
83. Macdougall IC, Cooper AC: Hyporesponsiveness to erythropoietic therapy due to chronic inflammation. Eur J Clin Invest 35:32-35, 2005 (suppl 3)
84. Barany P, Divino Filho JC, Bergstrom J: High C-reactive protein is a strong predictor of resistance to erythropoietin in hemodialysis patients. Am J Kidney Dis 29:565-568, 1997
85. Goicoechea M, Martin J, de Sequera P, et al: Role of cytokines in the response to erythropoietin in hemodialysis patients. Kidney Int 54: 1337-1343, 1998
86. Cooper AC, Mikhail A, Lethbridge MW, et al: Increased expression of erythropoiesis inhibiting cytokines (IFN-gamma, TNF-alpha, IL-10, and IL-13) by T cells in patients exhibiting a poor response to erythropoietin therapy. J Am Soc Nephrol 14:1776-1784, 2003
87. Schooley JC, Kullgren B, Allison AC: Inhibition by interleukin-1 of the action of erythropoietin on erythroid precursors and its possible role in the pathogenesis of hypoplastic anemias. Br J Haematol 67:11-17, 1987
88. Clibon U, Bonewald L, Caro J, et al: Erythropoietin fails to reverse the anemia in mice continuously exposed to tumor necrosis factor-alpha in vivo. Exp Hematol 18:438-441, 1990
89. Deicher R, Horl WH: New insights into the regulation of iron homeostasis. Eur J Clin Invest 36:301-309, 2006
90. Tomosugi N, Kawabata H, Wakatabe R, et al: Detection of serum hepcidin in renal failure and inflammation by using ProteinChip System(R). Blood e-Pub, April 2006
91. Kato H, Nangaku M, Fukamizu A, et al: Pathophysiological roles of renin-angiotensin system on erythropoietic action. Curr Hypertens Rev (in press)
92. Kato H, Ishida J, Imagawa S, et al: Enhanced erythropoiesis mediated by activation of the renin-angiotensin system via angiotensin II type 1a receptor. FASEB J 19:2023-2025, 2005
93. Keane WF, Lyle PA: Reduction of end points in NIDDM with the Angiotensin II Receptor Antagonist Losartan study. Recent advances in management of type 2 diabetes and nephropathy: Lessons from the RENAAL study. Am J Kidney Dis 41:S22-S25, 2003
94. Drueke TB, Eckardt KU: Role of secondary hyperparathyroidism in erythropoietin resistance of chronic renal failure patients. Nephrol Dial Transplant 17:28-31, 2002 (suppl 5)