Erythropoiesis, Erythropoietin, and Iron

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The basic components of erythropoiesis are stem cell precursors, their stimulation by erythropoietin, and an adequate supply of iron from which to make hemoglobin.

ANEMIA HAS LOST much of its mystique in the past 40 yr as a better understanding of erythropoiesis and red cell destruction has made diagnosis and management a more rational process. This brief discussion* will attempt to summarize certain physiologic concepts useful to the clinician.

The importance of erythrocyte size as an indicator of hemoglobin synthesis became evident with the introduction of the hematocrit over 50 yr ago, and anemias were classified as microcytic, normocytic, and macrocytic. Microcytosis could be equated with a decrease in hemoglobin synthesis, attributable to either iron deficiency, a mitochondrial abnormality affecting heme synthesis, or impaired globin synthesis. Macrocytosis is the result of a defect in nuclear maturation or of "stimulated erythropoiesis." The smear is essential in differentiating these two causes of red cell enlargement. True macrocytes are the result of a disruption of the usual mitotic sequence in the marrow (nuclear maturation defect). Cells that skip a mitosis appear as mature "red cell giants" in the circulating blood. A second type of macrocytosis is caused by increased erythropoietin stimulation in the presence of an adequate iron supply. In this normal physiologic response, hemoglobin synthesis within the immature cell is increased, and reticulocytes are released prematurely, appearing macrocytic, basophilic, and slightly hypochromic on blood smear. Automated equipment has increased the dependability of the mean corpuscular volume, but the interpretation of macrocytosis still rests with the smear differentiation of these two types of macrocytosis. Other calculations of red cell indices, i.e., mean corpuscular hemoglobin and hemoglobin concentration, add little.

There are limitations to this "cell size" approach. Changes take a long time to develop due to the slow turnover of circulating red cells, so that one can usually detect maturation abnormalities only after the condition has been present for months. Also, the relationship between iron supply and erythroid proliferation affects the degree of macrocytosis (Fig. 1). However, the real problem is that most anemias are normocytic and are therefore not amenable to this type of analysis.

The heterogeneous group of normocytic anemias can be best approached by measurements of production and destruction. Production is most simply quantitated by counting circulating reticulocytes as long as two corrections are made. The first is to convert the percent of circulating reticulocytes into their absolute number. The second adjusts for the effect of erythropoietin on the release of reticulocytes from the marrow. When plasma erythropoietin levels are increased, the usual reticulocyte maturation period in the marrow of about 3 days is shortened and the time in circulation is correspondingly lengthened (Fig. 2). The reticulo-
Fig. 1. Effect of erythropoietin stimulation and iron supply on erythrocyte size. Curves represent proposed changes in red cell volume at transferrin iron saturations from 10% to 100%. Normal marrow function is assumed. Increased erythropoietin stimulation in the presence of increased iron supply increases red cell hemoglobin and red cell size. As iron supply decreases, so will hemoglobin synthesis and red cell size. This relationship is also affected by abnormalities in hemoglobin synthesis, such as in thalassemia and by nuclear maturation abnormalities, which exaggerate the increase in hemoglobin synthesis by the iron-replete, stimulated erythroid precursor.

cyte index derived from these two corrections relates the rate of entry into circulation of the patient’s reticulocytes to basal production. The expected production capacity of the challenged erythroid marrow is about 5 times basal, an increase that can be achieved within a week under appropriate conditions. For practical purposes, a reticulocyte index of greater than 3 times basal is taken to indicate an adequate marrow response, while an index of less than 2 times basal is assumed to represent impaired production.

Production failure may be due to either inadequate erythroid proliferation or a maturation abnormality resulting in cell death during development (ineffective erythropoiesis). Although reticulocytes are low in each, the smear differentiates the two conditions (Fig. 3). A hypoproliferative marrow is usually associated with a fairly normal red cell population (a “quiet” smear), a low bilirubin, and a normal lactate dehydrogenase. Ineffective erythropoiesis will usually be detected by changes in mean corpuscular volume (increased with nuclear and decreased with cytoplasmic abnormalities), but is also associated with red cell fragmentation, a normal-to-high bilirubin, and an increased plasma lactate dehydrogenase. The erythroid:granulocyte (EG) ratio of the aspirated marrow or the density of erythroid forms in marrow section gives direct evidence of the degree of erythroid proliferation. Ferrokinetic measurements provide an even more accurate quantitation of the erythroid precursor number and are particularly useful when leukocyte abnormalities invalidate the EG ratio and when there is difficulty obtaining a representative marrow aliquot. This separation of anemias into hypoproliferative states, ineffective erythropoiesis, and hemolytic categories can serve as a scaffold for further differential diagnosis (Fig. 4). Such cataloging makes it clear that the vast majority of anemic patients have a hypoproliferative process in which erythropoietin and iron play prominent causative roles.

ERYTHROPOIETIN

Erythropoietin output is designed to modulate erythropoiesis so as to optimize blood hemoglobin concentration. This regulation is illustrated by the changes that follow altitude exposure, where the circulating hemoglobin concentration increases 1 g for each 3%–4% decrease in arterial oxygen concentration. However, regulation of oxygen supply is more complex than this. It has been observed that the hemoglobin concentration in patients with hemoglobinopathies correlated closely with the affinity of the abnormal hemoglobin for oxygen. Less complicated is the increase in hemoglobin concentration observed in patients with stable hemoglobin mutants having increased affinity for oxygen. These various observations indicate that the combination of O2 content and its availability rather than oxygen content of arterial blood alone is the basis for erythropoietin regulation.

The erythrocyte has an intrinsic mechanism for modifying oxygen release, based on its content of...
intraerythrocytic 2,3-diphosphoglycerate (DPG). As long as there is adequate phosphate substrate, the amount of erythrocyte DPG will be altered not only by changes in oxygen availability produced by an altered blood pH but also according to the proportion of oxygenated versus reduced hemoglobin in circulation. The compensatory nature of this response to hypoxia is shown by increases in DPG occurring in cardiac failure and anemia, making increased amounts of oxygen available at any given oxygen tension. Thus, two interlocking regulatory mechanisms involving the erythron exist, designed to ensure an adequate oxygen supply. DPG is the more rapidly responsive, acting within hours to improve available oxygen by increasing the amount release at any given oxygen tension. This "available oxygen," in turn, is monitored by the erythropoietin-regulating apparatus, and further adjustments in erythropoietin and erythropoiesis over a period of weeks are required to achieve an appropriate concentration of circulating hemoglobin.

The response of the erythroid marrow to anemia is initiated by the action of erythropoietin on committed stem cells (CFU-E). While it would be often helpful to know the level of stimulation in a patient, valid quantit-
tative measurements of erythropoietin have been limited to the research laboratory and have been virtually unavailable to the clinician. Recently, radioimmune assays have been developed that have biologic significance and may become generally available in the near future. However, a simple screen is the evaluation of the blood smear for the presence of "shift cells," since the premature release of these young reticulocytes is erythropoietin-dependent. In using this, one must be alert to stromal disorders that produce shift not because of increased erythropoietin, but rather because of disruption of marrow architecture.

Altered erythropoietin regulation occurs in pathologic states for different reasons. Production of erythropoietin is decreased by a variety of disorders impairing renal function, but differences between excretory and endocrine function of the kidney do occur. For example, in the hemolytic uremic syndrome, there is active stimulation of erythropoiesis despite nitrogen retention. A decrease in erythropoietin is seen with certain endocrine disorders and with protein-calorie malnutrition. In these situations, the erythropoietin-producing apparatus is intact, but output of the hormone is decreased, presumably because less oxygen is required for the altered metabolic state of the body. If erythropoietin is considered to be the regulator of red cell proliferation, it might seem surprising that the plasma erythropoietin concentration per se bears little relationship to the rate of erythropoiesis in most anemic patients. Near-normal levels are seen despite accelerated erythropoiesis in hereditary spherocytosis. From this we must assume that very little increase in erythropoietin level above basal is required to sustain the almost complete compensation in this hemolytic anemia. On the other hand, high levels of erythropoietin are usually found when production is depressed by some other abnormality, the highest level being present in aplastic anemia where stem cells are reduced or cannot proliferate. The usual cause of a discrepancy between marrow stimulation and response is an inadequate supply of iron.

IRON

Iron is distributed to body tissues by plasma transferrin. Recent studies show that the transferrin iron pool is not homogeneous but is composed of monoferric and diferric transferrin, the latter having a far greater capacity to dispense iron to tissue (Fig. 5). Since iron loading on transferrin is random, the amount of diferric iron is a function of the transferrin iron saturation. It was shown that this was the most meaningful expression of available iron, and further, that basal erythropoiesis could not be sustained when the proportion of diferric transferrin iron fell below 16%. A limitation of iron supply is characterized by an increase in the extraction of transferrin iron by the erythroid marrow from basal values of 5%-10% to a maximum of about 30% and a corresponding reduction in the 80 ± 20 min T½ radioiron clearance to about 20 min.

There are two outstanding abnormalities that characterize iron-deficient erythropoiesis. The impairment of hemoglobin synthesis, which eventually results in a hypochromic microcytic red cell, reflects the obligatory requirement of hemoglobin for iron. An impairment in viability of developing and mature red cells in iron deficiency has been suggested by iron kinetic studies and morphological evidence of cell fragmentation, but it is likely that the amount of cell wastage has been overstated. Of more relevance in explaining the anemia is the effect of iron deficiency on cellular proliferation. Transferrin iron has been shown to be essential for proliferation in in vitro cell cultures. Fetal tissues cannot develop in utero when maternal iron supply becomes severely restricted. Similarly, the proliferation of the erythroid marrow is restricted to near-basal levels when iron supply fails. The degree of disparity between plasma iron supply and erythropoietin-imposed demand is reflected in the degree of microcytosis and hypochromia of circulating red cells. For this reason, little microcytosis is seen when the patient with renal failure is iron deficient because erythropoietin levels are also suppressed.

Considerations of iron-deficient erythropoiesis have usually related the effect of a decreased iron supply to basal tissue iron requirements. The term "relative iron
'deficiency' designates an iron supply that would be adequate to meet the needs of basal erythropoiesis but which is not adequate to meet the needs of an expanded erythroid marrow. This relationship can be accurately monitored by measuring red cell protoporphyrin. An increased red cell protoporphyrin is found in patients with simple iron deficiency when transferrin saturation drops below 16%. In hemolytic states, an increase in protoporphyrin will be found at a normal transferrin saturation of 35% or at even higher saturations because of the increased marrow iron requirement. Previous-ly, reticulocytes were considered to be high in protoporphyrin, but it is now appreciated that any increase observed represented a relative iron deficiency. It is not uncommon to find an inadequate iron supply (high red cell protoporphyrin) in hemolytic anemia with production rates greater than 5 times basal.

At these high levels of erythropoiesis, transferrin procurement of iron becomes critical. Iron is made available to transferrin through recycling of red cell iron, through mobilization of iron stores, and through absorption. Little is known of the regulation of plasma iron supply except that the very act of giving up iron by transferrin appears to enhance the procurement of iron by transferrin. From existing sources, the normal individual has difficulty providing sufficient iron to support rates of erythropoiesis of greater than 3 times basal. Clinicians have learned to interpret high reticulocyte counts as indicating hemolysis. However, the only reason for this association is the large amount of iron made available in hemolytic anemia through the catabolism of damaged red cells and the corresponding increase in reticulocytes due to the effective erythropoiesis characteristic of hemolytic states. Similar degrees of reticulocytosis can be seen when blood loss occurs in an individual with parenchymal iron overload or when a near-saturated transferrin is maintained by large doses of oral and/or parenteral iron.

The critical nature of the plasma iron concentration in patients with hemolytic anemia may be demonstrated by ferrokinetic studies (Fig. 6). A decrease in transferrin saturation from 60% to 30%, as might be caused by inflammation, may halve the production of red cells. This is almost certainly due to a marrow iron flow insufficient to saturate erythroid membrane receptors for the transferrin iron complex. In the severely anemic patient, compensatory mechanisms are at work to maximize iron supply. Thus, iron absorption has been shown to be increased in thalassemia and there is an increased release of catabolized red cell iron from, rather than storage in, the macrophage. This results in a high plasma iron concentration, leading in turn to augmented erythropoiesis.

While this homeostatic mechanism may permit survival of the untransfused patient with thalassemia, it results in parenchymal iron overload in later years.

**THE ERYTHROID MARROW**

The target of both erythropoietin and iron is the committed stem cell population of the marrow. Experience with marrow transplant suggests that less than 5% of the total stem cell population is required to establish basal erythropoiesis, so that the potential proliferative capacity of the erythron is enormous. In considering the limitations imposed on the response to anemia, a major one would appear to be the bony confinement of the medullary cavity. Some additional space is created initially by the shift of marrow reticulocytes into the blood. Further increases can probably only be achieved through the gradual extension of active marrow into inactive medullary cavities. Such an expanded marrow with its augmented blood supply is seen in thalassemia, where erythroid proliferation may reach 10–15 times basal as measured by our current ferrokinetic techniques. This particular disorder has the advantage of small red cell precursors minimizing space problems and a decreased hemoglobin synthesis in individual cells reducing iron requirements. Nevertheless, there is evidence that so hyperplastic a marrow may have internal difficulties involving the disruption of sinusoidal walls with intermittent release of marrow into the blood and embolization to the lung. This level of marrow activity is also
poorly tolerated by the skeleton.\(^{37,39}\) There is severe osteopenia, and medullary cavities expand, at times allowing marrow to rupture through the bony encasement into the mediastinum. Thus, inescapable liabilities are associated with extreme marrow proliferation.

A host of other factors are involved in the intricate process of red cell production and maintenance, including structural features of the marrow bed, which permit orderly maturation and transsinusoidal migration, other aspects of stem cell activation, the provision of nutrients such as \(\text{B}_12\), folic acid, and pyridoxine so essential to red cell maturation, and various determinants of red cell survival in circulation. However, iron, erythropoietin, and committed erythroid stem cells remain a central triad in the determination of red cell production. An appreciation of the workings of these through relatively simply laboratory methods provides the clinician with a physiologic basis for understanding most anemias and for anticipating the secondary problems associated with disturbances in erythropoiesis.

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