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Review

Aging and erythropoiesis: Current state of knowledge

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ABSTRACT

Many studies now document the high prevalence of anemia in the elderly and its association with poor outcomes. Study of these anemic patients reveals that in most cases the underlying abnormality is diminished erythropoieis. This analysis outlines some of the salient observations underlying the evolution of current concepts of how aging impacts erythropoiesis, and suggests areas for future exploration and research.

Contents

Introduction

There are now many studies documenting the high prevalence of anemia in the elderly [1] and its association with poor health outcomes. Even mild degrees of anemia in men and women 65 and older have been associated with significant morbidity, including frailty [2], decreased bone density [3], decreased skeletal muscle strength and density [4], decreased physical performance [5] and decline in physical performance over time [6], and increased mortality [7,8]. Although these studies have not established causality, they do raise speculation as to the role that anemia plays in initiating or aggravating adverse clinical outcomes. While there is no uniformity of opinion regarding the importance or even existence of so-called "anemia of the aged", increasing attention has focused on its widespread prevalence and clinical importance, including an agenda-setting conference co-sponsored by both the National Institute of Aging (NIA) and the American Society of Hematology [9] to address a "public health crisis in hematology" [10]. This issue is particularly important in light of the

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aging population: by the year 2030, men and women age 65 and older are estimated to comprise almost 20% of the total United States population, reaching almost 71 million persons [11].

When analyzed according to classical hematologic practice, most of the anemias in this setting turn out to be caused by defective or deficient red blood cell production, i.e. diminished erythropoiesis. Thus it becomes important to try to understand how aging impacts erythropoiesis, and where age-related defects in erythropoiesis may arise. Age-related changes in erythropoiesis can broadly be classified into two general mechanistic categories: 1) alterations intrinsic to erythroid progenitor or hematopoietic stem cells and/or the local hematopoietic microenvironment, and 2) alterations in humoral control mechanisms, particularly related to secretion of the hormone erythropoietin and possible deterioration of hypoxia-sensing mechanisms, but also other changes in the endocrine milieu. Understanding how each of these impacts erythropoiesis in the aging human has evolved concomitantly with the tools that have been available over the past century.

Alterations intrinsic to erythroid progenitor or hematopoietic stem cells

Marrow cellularity

When evaluated by a standard morphological approach, a decrease in overall hematopoietic tissue in the bone marrow, with a concomitant increase in adipose tissue, has been well known to occur with aging [12,13]. However, these one-time static measurements, although lending themselves to speculation regarding important changes both in hematopoietic cellularity and the hematopoietic microenvironment, are potentially prone to sampling error. In addition, gross estimations of marrow cellularity do not provide any dynamic interpretation of underlying erythropoietic activity, nor do they provide a clear insight into underlying mechanisms. Thus, evaluation of marrow cellularity in a bone marrow specimen is a relatively crude tool that has little capacity to provide insight into age-related changes in erythropoiesis.

Kinetic measurements

While erythroid precursors are morphologically identifiable in the bone marrow (proerythroblasts, basophilic erythroblasts, polychromatophilic erythroblasts and orthochromic erythroblasts), early erythroid progenitors are not distinguishable by light microscopy. Thus, evaluation of early erythropoiesis has relied historically on indirect measurements. Ferrokinetic studies were first described in the 1950s [14] and include measurement of the rates of disappearance of iron from the plasma (used to calculate plasma iron turnover), and subsequent incorporation into red cells and their appearance into the circulation (red cell utilization) [15,16]. Clearance of iron from the plasma will be faster (thus the plasma iron turnover will be increased) in states of increased erythropoiesis, and slower (the plasma iron turnover will be decreased) in states of reduced erythropoiesis. Decreased red cell utilization suggests either ineffective or reduced erythropoiesis. Ferrokinetic studies have been considerably important in elucidating erythropoietic mechanisms and responses, and were used for example to illustrate the normal marrow's capacity to upregulate erythropoiesis 7–8 fold in the presence of adequate iron [17]. Models of iron kinetics are fairly complex, however [15,18], leading to a somewhat cumbersome instrument, and ferrokinetic studies are not currently in widespread use.

Few investigators have specifically evaluated erythropoiesis in the elderly using kinetic studies. In one study, red cell utilization of an oral iron dose was significantly decreased in the elderly [19], suggesting possible ineffective erythropoiesis. In a subsequent study by the same investigator, non-anemic elderly patients were found to have decreased red cell utilization when given oral iron; however, when intravenous iron was used in a second population, this difference was abolished [20]. In this second population, there was a mild increase in plasma iron turnover in the elderly subjects, possibly due to increased non-erythroid iron turnover. These findings led the investigators to suggest that elderly people may have increased liver retention of orally absorbed iron. Thus, kinetic studies have provided only limited information regarding the effects of aging on erythropoiesis. Perhaps most importantly, I discovered no studies using ferrokinetic methods that looked at the compensatory erythropoietic response to stress in elderly versus young humans.

In vitro clonogenic assays

Clonogenic hematopoietic progenitor assays have been used since the early 1960s [21] to characterize erythroid progenitor cells. These in vitro assays identify both the earlier burst-forming unit-erythroid (BFU-E) colonies and the later stage colony forming unit-erythroid (CFU-E) by their ability to give rise to hemoglobinized progeny. Studies of aging human marrows utilizing these assays have shown conflicting results. Thus, non-anemic elderly patients have been found to have both similar [22,23] and decreased [24] BFU-E and CFU-E frequencies compared to young anemic or non-anemic controls. Studies in anemic elderly adults have likewise been conflicting, demonstrating decreased BFU-E and CFU-E frequencies compared to young anemic controls [22], decreased BFU-E frequencies only in comparison to young non-anemic controls [24], and, in males, similar BFU-E but decreased CFU-E frequencies compared to a young male non-anemic control population [23]. Another study found increased BFU-E and CFU-E frequencies in elderly anemic and non-anemic women compared to elderly anemic and non-anemic men, without significant differences in elderly versus young controls [25]. Importantly, measurement of the number of BFU-E and CFU-E colonies in human marrow is not rigorously quantitative. Thus, colony frequency is a relative measurement. It is possible that the discrepant results seen in these studies are attributable to unreported differences in total marrow cellularity. The variability might also be attributable to differences in assay technique, and/or the populations studied. However, taken together, the available data are inconclusive regarding the impact of aging on erythroid progenitor cell number and/or function.

In addition, in recent years we have learned about the importance of the marrow stromal environment and its niches, and these studies have not provided any consistent evidence differentiating possible age-related intrinsic progenitor cell changes versus changes in the marrow environment.

Hematopoietic stem and progenitor cells: animal transplant studies

Significant advances have been made in the recent past in understanding hematopoietic stem and progenitor cell biology as it pertains to aging. Because of the need for quantitative as well as qualitative analysis, these studies have been difficult to perform in patients. However, studies using animal models have surprisingly shown an overall increase in frequency of hematopoietic stem cells with aging [26–29], whereas frequencies of common myeloid progenitors (CMPs) and megakaryocyte–erythrocyte progenitors (MEPs) were not increased in aged versus young mice [28]. Transplantation of young and old whole bone marrow into young recipients showed that old bone marrow provided increased numbers of donor-derived hematopoietic stem cells with long term multilineage reconstituting ability (LT-HSCs) [28], thereby suggesting that age-dependent expansion of this cell population was a cell intrinsic attribute. However, competitive transplant of LT-HSCs from young and old donors into young recipients revealed decreased overall donor-derived reconstitution from old compared to young donors, and in particular decreased donor-derived peripheral B lymphocytes, with preservation of myeloid reconstitution [28]. When young LT-HSCs

were transplanted into young and old recipients, the results were similar, again suggesting cell intrinsic rather than bone marrow microenvironment properties. Studies of global gene expression profiling of hematopoietic stem cells from old mice have revealed upregulation of genes involved in the inflammatory and stress responses [28,30], and downregulation of genes participating in chromatin regulation and DNA repair (reviewed in [31]). However, although these animal models have provided an important framework for trying to understand age-associated changes in the number and properties of hematopoietic stem cells and erythroid progenitor cells, such models are ultimately limited in that they may not fully replicate human biology.

Hematopoietic stem cells: human studies

Information regarding changes in stem cell number and function in humans is of particular interest in the clinical context of stem cell transplantation, and has been the subject of recent reviews [32–34]. Although the mobilization of progenitor cells in response to granulocyte-colony stimulating factor (G-CSF) may be decreased in elderly subjects [35], elderly transplant patients are generally able to donate adequate numbers of $CD34^+$cells for transplantation [32]. In the experimental setting, decreased circulating peripheral blood $CD34^+$ cells have been reported with aging [36]. In one study in which a $CD34^+$-enriched cell population was obtained from the peripheral blood, there were decreased $CD34^+$ cells in old (age 66 to 73 years) and very old (age 100 to 104 years) subjects compared to young (age 30 to 45 years) subjects [37]. However, there were no differences in frequencies of BFU-E grown in vitro from the $CD34^+$ cells collected in old versus young subjects. This group also found that there was decreased in vitro production of interleukin-3 (IL-3) and granulocyte-macrophage colony-stimulating factor (GM-CSF) by phytohaemagglutinin (PHA)-stimulated peripheral blood mononuclear cells from the very old group compared to the young group, and there was a gradual continuing increase in serum stem cell factor (SCF) levels with age. These data suggest that aging affects the number of circulating $CD34^+$ cells, and that there may be age-related changes in hematopoietic cytokine levels peripherally. However, in vivo analysis of hematopoietic stem and erythroid progenitor cell function outside of the clinical transplant setting has been limited, possibly in part by a relative poor availability of marrow specimens, and concerns about performing invasive biologic studies in elderly individuals. Nor have erythroid progenitors upstream of BFU-E and CFU-E been explored with any depth in the human. Thus there are large gaps in our knowledge regarding possible age-related changes in human hematopoietic stem and erythroid progenitor cells, and/or the human marrow microenvironment.

A summary of the impact of aging on stem cells, progenitor cells, and the marrow microenvironment is listed in Table 1.

Table 1
The impact of aging on erythropoiesis: hematopoietic stem and erythroid progenitor cells and the bone marrow microenvironment

Observation	Reference
Marrow cellularity	
Decreased marrow cellularity in the elderly	[12,13]
Ferrokinetic studies	
Decreased red cell uptake with oral but not intravenous iron in elderly versus young subjects	[19,20]
Clonogenic Assays	
Non-anemic Elderly	
Decreased BFU-E and CFU-E frequencies compared to young controls	[24]
Similar BFU-E and CFU-E frequencies compared to young controls	[22,23]
Anemic Elderly	
Decreased BFU-E and CFU-E frequencies compared to young anemic controls	[22]
Decreased BFU-E but similar CFU-E frequencies compared to young controls	[24]
Decreased CFU-E but similar BFU-E frequencies in males compared to controls	[23]
Men versus women	
Increased BFU-E and CFU-E frequencies in elderly anemic and non-anemic women compared to men but no differences in elderly versus young controls	
Hematopoietic stem and progenitor cells: animal models	
Increase in hematopoietic stem cell frequency with aging	[26–29]
Similar common myeloid progenitor and megakaryocyte-erythrocyte progenitor frequencies in elderly versus young mice	[28]
Upregulation of genes involved in inflammatory and stress responses with aging	[28,30]
Downregulation of genes involved in chromatin regulation and DNA repair	[31]
Hematopoietic stem cells: human studies	
Decreased circulating CD34+ cells in the elderly	[36,37]
Increased serum stem cell factor levels in the elderly	[37]

Alterations in humoral control mechanisms

Erythropoietin levels

In adults, erythropoietin is produced primarily in the kidneys, and acts on the erythroid progenitors to prevent cell death [38,39]. Basal erythropoietin levels are approximately 10-20 mU/ml [40] and the normal response to decreased oxygen tension in the blood is a logarithmic increase in erythropoietin levels upwards of 1000-fold over basal levels in the case of severe anemia [41]. In contrast, patients with renal disease have a blunted erythropoietin response to anemia [42], and worsening renal excretory function corresponds with lower erythropoietin levels [43–45].

Cross-sectional studies of erythropoietin levels in elderly non-anemic subjects have shown conflicting results, with some studies finding no significant difference in erythropoietin levels compared to young non-anemic controls [46–48], whereas others have found that erythropoietin levels are significantly increased in the elderly compared to young controls [49,50]. One intriguing longitudinal analysis of 143 adults followed for at least 8 years predicted a modest but steady decline in hemoglobin levels over time, with the majority of participants remaining within the normal range for the duration of the study, and a concomitant modest but steady increase in erythropoietin levels [51].

Studies regarding erythropoietin levels in elderly anemic subjects are also conflicting, and may be confounded by the known decease in renal excretory function that accompanies aging [52]. In one study of 71 patients who donated autologous blood prior to surgery, the slope of the log erythropoietin versus hemoglobin did not differ in those over versus those under 65 years of age [53]. Measures of renal function were not reported in this study. In contrast, in another study in patients with iron deficiency anemia, those 74 years and older had lower erythropoietin levels compared to adults under 60 in response to similar degrees of anemia [54]. Although patients with a serum creatinine > 120 μmol/ml (1.4 mg/dL) were excluded from this study, the mean calculated creatinine clearance using the Cockcroft–Gault equation was 44 ml/min/1.73 m^2 in the elderly group, and decreased with increasing age, suggesting that the reduced erythropoietin response might correspond at least in part to decreased renal excretory function.

Erythropoietin levels may also be reduced in elderly patients with so-called "unexplained anemia" of aging, that is, anemia in which no etiology can be found. Those with unexplained anemia are of particular interest, as they may reveal primary age-associated defects in erythropoiesis. In one study, although the mean erythropoietin level in elderly subjects with unexplained anemia was significantly increased compared to elderly non-anemic subjects, it was lower than the mean erythropoietin level seen in those with iron deficiency anemia (29.5 mU/ml versus 72.4 mU/ml) [50]. In addition, although in both younger and older subjects with iron deficiency anemia there was a significant inverse correlation between hemoglobin concentration and erythropoietin levels, no such correlation was seen in those with unexplained

anemia. Erythropoietin levels may also be decreased in states of malnutrition. In one study, elderly subjects who were malnourished had a blunted erythropoietin response to anemia [48]. These findings suggest that decreased erythropoietin plays a role in the pathogenesis of both unexplained anemia of the elderly and anemia due to malnutrition.

Altered erythropoietin response may be mediated by the co-morbidities of diabetes and hypertension, both common diseases in the elderly [55,56]. Although there are reports to the contrary [57], a growing body of evidence suggests that hypertension and particularly diabetes are associated with decreased erythropoietin levels [51,58–71]. In one study, erythropoietin levels in anemic diabetics without renal disease (defined as a serum creatinine ≥ 1.6 g/dL or calculated creatinine clearance < 50 ml/min/1.73 m^2) were significantly less compared to levels in non-diabetics across all etiologies of anemia except for myeloproliferative disorders and megaloblastic anemias [72]. Potential underlying pathologic mechanisms of reduced erythropoietin levels in patients with diabetes and/or hypertension include the loss of hypoxic response as a stimulus for erythropoietin secretion, possibly due to functional and/or structural changes in the proximal tubule and cortical interstitium [73,74], effects of advanced glycation end products [75], or autonomic dysfunction [76–78].

Taken together, these studies, although mixed, suggest that aging may be accompanied by a relative erythroid resistance to erythropoietin, leading to a need for increased erythropoietin secretion in order to maintain the red cell mass at physiologic levels. When this feedback loop is interrupted, particularly in those with renal disease, diabetes or hypertension, erythropoietin secretion diminishes, and anemia results. This would also explain why in elderly adults with iron deficiency alone in whom there is no disruption of this physiologic process, there would be an erythropoietin response comparable to that seen in younger anemic controls. Alternative explanations for increased erythropoietin levels in non-anemic elderly subjects include decreased clearance due to reduced numbers of erythropoietin receptors in a relatively hypoplastic marrow, or an increase in the hypoxic stimulus for erythropoietin production.

Hypoxia-inducible factor (HIF) and aging

Regulation of erythropoietin is complex (reviewed in [79] and [76]) with decreased tissue oxygen tension serving as the primary stimulus for erythropoietin secretion. A major breakthrough in understanding occurred in the early 1990s with the elucidation of control of erythropoietin gene expression by the hypoxia-inducible factor (HIF) family of transcription factors ([80] and reviewed in [81]). There are three main isoforms of HIF-α (HIF-1α, HIF-2α and HIF-3α). Under normoxic conditions, HIF-α is hydroxylated at key proline residues by a series of oxygen dependent iron-requiring prolyl hydroxylases (PHDs) followed by binding of the von Hippel Lindau tumor-suppressor protein, leading to ubiquitination and degradation in the proteasome [82,83]. Under hypoxic conditions, HIF-α is not hydroxylated, and instead rapidly enters the nucleus, and binds to HIF-1β. The heterodimeric complex of HIF-α and HIF-1β binds to a conserved DNA motif, the hypoxia response element (HRE), located in a 3' enhancer region of the erythropoietin gene which acts synergistically with the promoter to initiate *de novo* transcription of erythropoietin [82,84–86]. HIF transcriptional activity is also modulated via hydroxylation of asparagine residues, which sterically block the binding of transcriptional coactivators [81]. It is not yet known whether HIF-1α or HIF-2α is the primary regulator of erythropoietin transcription in the kidney, although animal models suggest that HIF-2α primarily regulates hepatic erythropoietin expression [87].

The effect of aging on erythropoietin synthesis was examined in an animal model using rats harboring a transgene composed of HRE and a reporter gene. In comparison to young rats, aged rats had both higher creatinine levels and degrees of proteinuria, and higher degrees of tubulo-interstitial injury, which correlated with hypoxia as measured by transgene expression [88]. Erythropoietin and VEGF mRNA levels were also higher in the aged kidneys. This finding suggests increased renal hypoxia in aged rat kidneys as a stimulus for increased erythropoietin production.

There are in vitro and animal model data suggestive of altered HIF responses to hypoxia with aging. In one study, carotid bodies from old rats had higher levels of HIF-1α measured by immunohistochemistry at baseline, but a blunted increase in response to intermittent hypoxia in comparison to carotid bodies from young rats [89]. Decreased levels of HIF-1α expression by immunohistochemistry have also been seen in vitro in hypoxia-challenged vascular smooth muscle cells from old rabbits compared to young rabbits [90]. In another study, there were decreased HIF–HRE complexes as measured by electromobility shift assays in brain and liver of aged versus young mice, whereas the quantity of HIF-1α protein was not correspondingly decreased, suggesting reduced HIF–HRE binding [91]. Thus, these animal studies suggest that aging, while possibly associated with an increase in local tissue hypoxia, may also bring with it a diminished ability to respond to hypoxia. It is not known whether there are age-related changes in the response to hypoxia that affect erythropoieis in man.

Other endocrine influences

Changes in endocrine function with aging are diverse [92] and include decreased testosterone [93–95] and growth hormone levels. Androgens may play a role in erythropoiesis, and have been used pharmacologically in the treatment of anemia due to renal disease [96–98], although this use has largely been supplanted by the use of recombinant human erythropoietin. The mechanism of improvement in anemia is likely due in part to increased erythropoietin levels [99,100]. However, in one study, increases in erythropoietin levels did not correlate with hemoglobin response to androgen therapy [101], suggesting additional erythropoietic mechanisms, which may include increased stem cell cycling [102]. Additional evidence suggesting a role for androgens in erythropoiesis includes the development of anemia when anti-androgen therapy is used in the treatment of prostate cancer [103,104], particularly when combined hormonal blockade, utilizing both a competitive inhibitor of the androgen receptor and surgical or chemical castration, is used [105].

The question of whether low testosterone levels in the elderly impact erythropoiesis was explored in one study in which testosterone levels were measured in 905 men and women 65 and older who were not undergoing treatment with anti-androgen therapy and who did not have cancer or renal insufficiency. In this study, men and women in the lowest quartile of total and bioavailable testosterone levels were more likely than those in the highest quartile to have anemia, and also were more likely to develop anemia over time [106]. However, only a minority of men and women in the lowest quartiles had anemia, suggesting that low testosterone in itself is not sufficient to cause anemia. One possible explanation is that low testosterone may act as an additive insult, potentially driving susceptible elderly adults into the anemic range. More studies are needed to further explore the impact of low testosterone levels on erythropoiesis in the elderly. Whether to treat elderly men and women with exogenous testosterone in order to correct anemia is also unknown, particularly given the potential risks of such treatment [107,108].

The secretion of growth hormone also decreases with older age [109,110]. The effects of growth hormone on erythropoiesis appear to be mediated through insulin-like growth factor-I [111] and include inhibition of apoptosis [112,113] and potentiation of the effects of erythropoietin [114] and other cytokines. Growth hormone may increase erythropoietin levels, possibly due to increased hepatic synthesis of erythropoietin [115]. Growth hormone replacement ameliorates anemia in hypophysectized rats [116], growth hormone deficient children [117], and growth hormone deficient adults [118]. Growth hormone may also affect the plasma volume. In one study,

administration of growth hormone to growth hormone deficient adults led to increases in the red cell mass with decreases in mean corpuscular volume, mean corpuscular hemoglobin and serum ferritin levels, although the hemoglobin concentration did not significantly change [119]. The impact of diminished growth hormone on erythropoiesis in the elderly and its possible role in the development of anemia require further study.

Inflammation and erythropoiesis

Inflammation has long been known to affect erythropoiesis, perhaps most profoundly in the "anemia of chronic inflammation" (ACI), also known as "anemia of chronic disease" (ACD). ACI is characterized typically as a normocytic, normochromic anemia with slightly shortened red cell survival, reduced erythropoietin levels, and a blunted response to erythropoietin. Recent investigation has opened up many insights into the pathophysiology of ACI, particularly with regard to the iron-modulating peptide hepcidin, which are well described elsewhere [120,121] and are beyond the scope of this review. In brief summary, it is now known that inflammatory mediators, particularly interleukin-6 (IL-6) and interleukin-1 (IL-1), lead to increased hepatic synthesis of hepcidin. Hepcidin in turn acts to prevent absorption of iron from the gut and release of iron from macrophages in the bone marrow to erythroid progenitors and precursors [122].

Elderly subjects are consistently found to have increased levels of inflammatory mediators [123,124], and in particular IL-6 levels [125] (and reviewed in [126]). Importantly, this increase tends to be relatively mild, that is, a low-level chronic increase in inflammatory mediators, as opposed to the dramatically elevated inflammatory state which can be seen in acute illness or auto-immune diseases. Also of importance, the increased inflammation found in the elderly may be due at least in part to associated co-morbidities rather than a property intrinsic to the aging process itself. In one study, age-related increases in inflammatory markers, including IL-6, were substantially reduced when the analysis was adjusted for cardiovascular and other diseases [127].

Studies on the association of inflammation with anemia in the elderly have shown mixed results. In one study, IL-6, tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP) levels were all significantly increased in elderly anemic versus non-anemic individuals [5], and in another study elderly anemic participants had higher IL-6, TNFα, CRP and IL-1 levels compared to non-anemic elderly participants [128]. In addition, those who were anemic and had the highest number of inflammatory markers in the upper tertile had significantly lower erythropoietin levels than those with the lowest number of inflammatory markers in the upper tertile [128], suggesting ACI-like pathophysiology. In contrast, in another study IL-6 levels in elderly anemic subjects tended to be lower than in non-anemic subjects, although this difference was not statistically significant [129].

It is possible that inflammation may lead to otherwise unexplained anemia of the elderly. However, in contradiction of this hypothesis, in one study elderly patients with unexplained anemia had no differences in IL-6, TNF-α, or CRP levels compared to a non-anemic elderly control population [130]. Unexplained anemia was defined as the absence of renal disease (defined as a creatinine clearance calculated from a 24 hour urine collection of less than 0.5 ml/s), the absence of iron deficiency (based on measurements of the soluble transferrin receptor and serum ferritin levels), the absence of ACI (defined as low serum iron without evidence of iron deficiency), and the absence of B12 and folate deficiency. It may be that these purely laboratory-based measurements do not allow for fine-tuning of categorization. However, these results argue against a role for inflammation in the development of otherwise unexplained anemia in the elderly. Further studies are needed to better understand the impact of inflammation, including the role of hepcidin, on erythropoiesis in the elderly human.

Table 2
The impact of aging on erythropoiesis: humoral control mechanisms

Observation	Reference
Erythropoietin (epo) levels	
In non-anemic elderly	
Similar epo levels in old versus young non-anemic subjects	[46–48]
Increased epo levels in old versus young non-anemic subjects	[49,50]
Increased epo levels with aging over time	[51]
In anemic elderly	
Similar epo levels in response to blood donation in old versus young subjects	[53]
Decreased epo levels in old versus young subjects with iron deficiency anemia	[54]
Decreased epo levels in old subjects with unexplained anemia versus those with iron deficiency anemia	[50]
Decreased epo levels in elderly subjects with malnutrition	[48]
In those with diabetes and/or hypertension	
Similar epo levels in subjects with diabetes versus those without diabetes	[57]
Decreased epo levels in subjects with diabetes and/or hypertension versus those without diabetes and/or hypertension	[51,58–72]
Hypoxia-inducible factor and aging: animal studies	
Increased hypoxia, tubulo-interstitial injury and epo mRNA in aged versus young rat kidneys	[88]
Increased HIF-1α levels as measured by immunohistochemistry in carotid bodies from old rats compared to young rats	[89]
Decreased HIF-1α response as measured by immunohistochemistry in hypoxic challenge models	[89,90]
Decreased HIF-HRE complexes by electromobility shift assays in brain and liver of old versus young mice with stable HIF-1 protein levels	[91]
Changes in endocrine function with aging	
Elderly men and women with the lowest testosterone levels more likely to have anemia or develop anemia over time	[106]
Inflammation and erythropoiesis and aging	
Increased levels of inflammatory mediators in elderly subjects	[123–126]
Increased levels of inflammatory mediators in anemic versus non-anemic elderly subjects	[5,128]
Similar levels of inflammatory mediators in anemic versus non-anemic elderly subjects	[129]

A summary of the effects of aging on humoral control mechanisms are listed in Table 2.

Summary

The impact of aging on erythropoiesis can be viewed in the context of two general mechanistic categories: 1) alterations intrinsic to hematopoietic stem or erythroid progenitor cells and/or the local hematopoietic microenvironment, and 2) alterations in humoral control mechanisms. Despite major advances in understanding of human hematopoietic stem and erythroid progenitor cell biology in the past century, the available data regarding the impact of aging on these cell populations remain conflicting and/or sparse. Animal studies suggest that aging is associated with expansion of hematopoietic stem cells, a property that is transplantable and thus intrinsic to the aging stem cells. In contrast, in mice CMP and MEP frequencies do not appear to change dramatically with aging. Similar data are lacking in humans. Data in humans regarding age-related changes in BFU-E and CFU-E are conflicting, and not reliably quantitative. Further, there are no human data available regarding erythroid progenitors upstream of BFU-E and CFU-E. Thus, new approaches are needed to explore cellular reservoirs potentially impacted by aging.

Knowledge regarding age-related alterations in humoral control mechanisms is also limited. Many, but not all, studies suggest that erythropoietin levels rise with aging; however, potential underlying mechanisms are not well understood, including the impact of aging on the global response to hypoxia. Nor do we have a good understanding of how changes in testosterone, growth hormone, or inflammation in the aging human affect erythropoiesis.

Areas for future exploration

Potential areas for further exploration include the quantitative evaluation of hematopoietic stem cells, common myeloid progenitors and megakaryocyte–erythrocyte progenitors in young non-anemic, elderly non-anemic and elderly anemic populations. Elderly anemic populations could further be analyzed according to the etiology of the anemia, with a focus on those with unexplained anemia, who are most likely to demonstrate age-related defects in erythropoiesis. Careful studies could provide essential information as to how aging impacts each of these cellular compartments, and what derangements are seen with various age-associated anemias.

Studying how the erythroid response to stress changes with aging could also provide vital information. While Finch [15] and Hillman[17] have provided useful clinical information on the level of compensatory erythropoietic activity in the anemically stressed normal adult, we have no comparable information on the similarly stressed elderly adult. The soluble transferrin receptor, which is elevated in states of increased erythropoiesis [131], could be a useful tool in this setting. Further studies evaluating the impact of aging on the human bone marrow microenvironment are critically important, given the morphologic changes in cellularity and adipose tissue, however definitive experiments on microenvironmental factors in humans remain difficult to design and perform.

Perhaps equally important, the impact of possible changes in plasma volume needs to be addressed. Both hemoglobin and hematocrit are concentration measurements, and thus subject to both expansion and contraction of the plasma volume. Elderly patients with heart failure may have plasma volume expansion, and thus a normal red cell mass despite a decreased hematocrit. In contrast, those on diuretic therapy may have a contracted plasma volume, masking either the presence or the degree of anemia. This in part could explain why even mild anemia has been associated in epidemiologic studies with poor outcomes. Red cell mass and plasma volume measurements in elderly non-anemic and anemic subjects will be critical in sorting this out.

Regarding the hormonal changes associated with aging, more information is needed to further elucidate possible age-related changes in erythropoietin levels, and whether, if present, such changes are due to altered hypoxia-sensing mechanisms, increased hypoxia, resistance of erythroid progenitors to erythropoietin, or alterations in the half life and/or glycosylation of erythropoietin.

Thus, although progress has been made in better understanding how aging impacts erythropoiesis, further work is needed to more completely understand these variable processes, and, importantly, how possible derangement of the underlying physiology may lead to anemia in the elderly, and its important clinical consequences.

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References

[1] J.M. Guralnik, R.S. Eisenstaedt, L. Ferrucci, et al., Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia, Blood 104 (2004) 2263–2268.
[2] P.H. Chaves, R.D. Semba, S.X. Leng, et al., Impact of anemia and cardiovascular disease on frailty status of community-dwelling older women: the Women's Health and Aging Studies I and II, J. Gerontol., A, Biol. Sci. Med. Sci. 60 (2005) 729–735.
[3] M. Cesari, M. Pahor, F. Lauretani, et al., Bone density and hemoglobin levels in older persons: results from the InCHIANTI study, Osteoporos. Int. 16 (2005) 691–699.
[4] M. Cesari, B.W. Penninx, F. Lauretani, et al., Hemoglobin levels and skeletal muscle: results from the InCHIANTI study, J. Gerontol., A, Biol. Sci. Med. Sci. 59 (2004) 249–254.
[5] B.W. Penninx, M. Pahor, M. Cesari, et al., Anemia is associated with disability and decreased physical performance and muscle strength in the elderly, J. Am. Geriatr. Soc. 52 (2004) 719–724.
[6] B.W. Penninx, J.M. Guralnik, G. Onder, et al., Anemia and decline in physical performance among older persons, Am. J. Med. 115 (2003) 104–110.
[7] N.A. Zakai, R. Katz, C. Hirsch, et al., A prospective study of anemia status, hemoglobin concentration, and mortality in an elderly cohort: the Cardiovascular Health Study, Arch. Intern. Med. 165 (2005) 2214–2220.
[8] P.H. Chaves, Q.L. Xue, J.M. Guralnik, et al., What constitutes normal hemoglobin concentration in community-dwelling disabled older women? J. Am. Geriatr. Soc. 52 (2004) 1811–1816.
[9] S.L. Schrier, Hematology, ASH, and the anemia of the aged, Blood 106 (2005) 3341–3342.
[10] J.M. Guralnik, W.B. Ershler, S.L. Schrier, V.J. Picozzi, Anemia in the elderly: a public health crisis in hematology, Hematol. Am. Soc. Hematol. Educ. Program (2005) 528–532.
[11] U.S. Interim Projections by Age, Sex, Race, and Hispanic Origin [http://www.census.gov/ipc/www/usinterimproj/natprojtab02a.pdf].
[12] R.J. Hartsock, E.B. Smith, C.S. Petty, Normal variations with aging of the amount of hematopoietic tissue in bone marrow from the anterior iliac crest. A study made from 177 cases of sudden death examined by necropsy, Am. J. Clin. Pathol. 43 (1965) 326–331.
[13] J. Justesen, K. Stenderup, E.N. Ebbesen, et al., Adipocyte tissue volume in bone marrow is increased with aging and in patients with osteoporosis, Biogerontology 2 (2001) 165–171.
[14] R.L. Huff, T.G. Hennessy, R.E. Austin, et al., Plasma and red cell iron turnover in normal subjects and in patients having various hematopoietic disorders, J. Clin. Invest. 29 (1950) 1041–1052.
[15] C.A. Finch, K. Deubelbeiss, J.D. Cook, et al., Ferrokinetics in man, Medicine (Baltimore) 49 (1970) 17–53.
[16] F. Hosain, G. Marsaglia, C.A. Finch, Blood ferrokinetics in normal man, J. Clin. Invest. 46 (1967) 1–9.
[17] R.S. Hillman, P.A. Henderson, Control of marrow production by the level of iron supply, J. Clin. Invest. 48 (1969) 454–460.
[18] C. Ricketts, I. Cavill, J.A. Napier, A. Jacobs, Ferrokinetics and erythropoiesis in man: an evaluation of ferrokinetic measurements, Br. J. Haematol. 35 (1977) 41–47.
[19] J.J. Marx, Normal iron absorption and decreased red cell iron uptake in the aged, Blood 53 (1979) 204–211.
[20] J.J. Marx, H.J. Dinant, Ferrokinetics and red cell iron uptake in old age: evidence for increased liver iron retention? Haematologica 67 (1982) 161–168.
[21] T.R. Bradley, D. Metcalf, The growth of mouse bone marrow cells in vitro, Aust. J. Exp. Biol. Med. Sci. 44 (1966) 287–299.
[22] C.A. Baraldi-Junkins, A.C. Beck, G. Rothstein, Hematopoiesis and cytokines. Relevance to cancer and aging, Hematol. Oncol. Clin. North. Am. 14 (2000) 45–61 viii.
[23] D.A. Lipschitz, K.B. Udupa, K.Y. Milton, C.O. Thompson, Effect of age on hematopoiesis in man, Blood 63 (1984) 502–509.
[24] Y. Hirota, S. Okamura, N. Kimura, et al., Haematopoiesis in the aged as studied by in vitro colony assay, Eur. J. Haematol. 40 (1988) 83–90.
[25] H. Nilsson-Ehle, B. Swolin, J. Westin, Bone marrow progenitor cell growth and karyotype changes in healthy 88-year-old subjects, Eur. J. Haematol. 55 (1995) 14–18.
[26] K. Sudo, H. Ema, Y. Morita, H. Nakauchi, Age-associated characteristics of murine hematopoietic stem cells, J. Exp. Med. 192 (2000) 1273–1280.
[27] S.J. Morrison, A.M. Wandycz, K. Akashi, et al., The aging of hematopoietic stem cells, Nat. Med. 2 (1996) 1011–1016.
[28] D.J. Rossi, D. Bryder, J.M. Zahn, et al., Cell intrinsic alterations underlie hematopoietic stem cell aging, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 9194–9199.
[29] D.J. Pearce, F. Anjos-Afonso, C.M. Ridler, et al., Age-dependent increase in side population distribution within hematopoiesis: implications for our understanding of the mechanism of aging, Stem Cells 25 (2007) 828–835.
[30] S.M. Chambers, C.A. Shaw, C. Gatza, et al., Aging hematopoietic stem cells decline in function and exhibit epigenetic dysregulation, PLoS Biol. 5 (2007) e201.
[31] S.M. Chambers, M.A. Goodell, Hematopoietic stem cell aging: wrinkles in stem cell potential, Stem Cell Rev. 3 (2007) 201–211.
[32] G. Ballester, M.T. Tirona, O. Ballester, Hematopoietic stem cell transplantation in the elderly, Oncology (Williston. Park) 21 (2007) 1576–1583. Discussion 1587, 1590–1571, 1606.
[33] L. Balducci, C.L. Hardy, G.H. Lyman, Hemopoiesis and aging, Cancer Treat. Res. 124 (2005) 109–134.
[34] L. Berkahn, A. Keating, Hematopoiesis in the elderly, Hematology 9 (2004) 159–163.
[35] G.S. Chatta, T.H. Price, R.C. Allen, D.C. Dale, Effects of in vivo recombinant methionyl human granulocyte colony-stimulating factor on the neutrophil response and peripheral blood colony-forming cells in healthy young and elderly adult volunteers, Blood 84 (1994) 2923–2929.
[36] Y. Egusa, Y. Fujiwara, E. Syahruddin, et al., Effect of age on human peripheral blood stem cells, Oncol. Rep. 5 (1998) 397–400.
[37] G.P. Bagnara, L. Bonsi, P. Strippoli, et al., Hemopoiesis in healthy old people and centenarians: well-maintained responsiveness of CD34+cells to hemopoietic growth factors and remodeling of cytokine network, J. Gerontol., A, Biol. Sci. Med. Sci. 55 (2000) B61–B66. Discussion B67–70.
[38] M.J. Koury, M.C. Bondurant, Erythropoietin retards DNA breakdown and prevents programmed death in erythroid progenitor cells, Science 248 (1990) 378–381.
[39] G.D. Longmore, S.S. Watowich, D.J. Hilton, H.F. Lodish, The erythropoietin receptor: its role in hematopoiesis and myeloproliferative diseases, J. Cell Biol. 123 (1993) 1305–1308.
[40] A.J. Erslev, Erythropoietin, N. Engl. J. Med. 324 (1991) 1339–1344.
[41] A.J. Erslev, J. Caro, O. Miller, R. Silver, Plasma erythropoietin in health and disease, Ann. Clin. Lab. Sci. 10 (1980) 250–257.

[42] J.W. Adamson, J. Eschbach, C.A. Finch, The kidney and erythropoiesis, Am. J. Med. 44 (1968) 725–733.
[43] H.W. Radtke, A. Claussner, P.M. Erbes, et al., Serum erythropoietin concentration in chronic renal failure: relationship to degree of anemia and excretory renal function, Blood 54 (1979) 877–884.
[44] F. Artunc, T. Risler, Serum erythropoietin concentrations and responses to anaemia in patients with or without chronic kidney disease, Nephrol. Dial. Transplant. 22 (2007) 2900–2908.
[45] M. Vogeser, X. Schiel, Serum erythropoietin concentrations in patients with anemia-preliminary hemoglobin-related reference ranges, Clin. Lab. 48 (2002) 595–598.
[46] M. Mori, Y. Murai, M. Hirai, et al., Serum erythropoietin titers in the aged, Mech. Ageing Dev. 46 (1988) 105–109.
[47] C.G. Musso, C.A. Musso, H. Joseph, et al., Plasma erythropoietin levels in the oldest old, Int. Urol. Nephrol. 36 (2004) 259–262.
[48] J.S. Powers, M.J. Lichtenstein, J.C. Collins, et al., Serum erythropoietin in healthy older persons, J. Am. Geriatr. Soc. 37 (1989) 388–389.
[49] K. Kario, T. Matsuo, K. Nakao, Serum erythropoietin levels in the elderly, Gerontology 37 (1991) 345–348.
[50] K. Kario, T. Matsuo, K. Kodama, et al., Reduced erythropoietin secretion in senile anemia, Am. J. Hematol. 41 (1992) 252–257.
[51] W.B. Ershler, S. Sheng, J. McKelvey, et al., Serum erythropoietin and aging: a longitudinal analysis, J. Am. Geriatr. Soc. 53 (2005) 1360–1365.
[52] J.W. Rowe, R. Andres, J.D. Tobin, et al., The effect of age on creatinine clearance in men: a cross-sectional and longitudinal study, J. Gerontol. 31 (1976) 155–163.
[53] L.T. Goodnough, T.H. Price, C.A. Parvin, The endogenous erythropoietin response and the erythropoietic response to blood loss anemia: the effects of age and gender, J. Lab. Clin. Med. 126 (1995) 57–64.
[54] J. Nafziger, K. Pailla, L. Luciani, et al., Decreased erythropoietin responsiveness to iron deficiency anemia in the elderly, Am. J. Hematol. 43 (1993) 172–176.
[55] N. NIDDK: http://diabetes.niddk.nih.gov/dm/pubs/statistics/#9. In: National Diabetes Information Clearinghouse. 2006.
[56] Racial/Ethnic Disparities in Prevalence, Treatment and Control of Hypertension—United States, 1999–2002, MMWR, vol. 54, 2005, pp. 7–9.
[57] C.M. Bruno, C. Sciacca, G. Bertino, et al., Circulating erythropoietin in microalbuminuric type 2 diabetic patients with normal renal function: a pilot study, J. Diabetes its Complicat. 20 (2006) 376–379.
[58] S.H. Ahn, H.S. Garewal, Low erythropoietin level can cause anemia in patients without advanced renal failure, Am. J. Med. 116 (2004) 280–281.
[59] A.S. Winkler, J. Marsden, K.R. Chaudhuri, et al., Erythropoietin depletion and anaemia in diabetes mellitus, Diabet. Med. 16 (1999) 813–819.
[60] D.R. Bosman, A.S. Winkler, J.T. Marsden, et al., Anemia with erythropoietin deficiency occurs early in diabetic nephropathy, Diabetes Care 24 (2001) 495–499.
[61] Y.S. Yun, H.C. Lee, N.C. Yoo, et al., Reduced erythropoietin responsiveness to anemia in diabetic patients before advanced diabetic nephropathy, Diabetes Res. Clin. Pract. 46 (1999) 223–229.
[62] M.U. Rarick, B.M. Espina, D.T. Colley, et al., Treatment of a unique anemia in patients with IDDM with epoetin alfa, Diabetes Care 21 (1998) 423–426.
[63] A.M. Kallab, G. Dabaghian, T. Terjanian, Anemia secondary to low erythropoietin in a patient with normal renal function, Mt. Sinai J. Med. 64 (1997) 406–408.
[64] P. Cotroneo, B. Maria Ricerca, L. Todaro, et al., Blunted erythropoietin response to anemia in patients with Type 1 diabetes, Diabetes Metab. Res. Rev. 16 (2000) 172–176.
[65] S. Hadjadj, F. Torremocha, A. Fanelli, et al., Erythropoietin-dependent anaemia: a possible complication of diabetic neuropathy, Diabetes Metab. 27 (2001) 383–385.
[66] K. Kojima, Y. Totsuka, Anemia due to reduced serum erythropoietin concentration in non-uremic diabetic patients, Diabetes Res. Clin. Pract. 27 (1995) 229–233.
[67] R. Ravanan, J.R. Spiro, P.W. Mathieson, R.M. Smith, Impact of diabetes on haemoglobin levels in renal disease, Diabetologia 50 (2007) 26–31.
[68] O.A. Mojiminiyi, N.A. Abdella, M.Y. Zaki, et al., Prevalence and associations of low plasma erythropoietin in patients with Type 2 diabetes mellitus, Diabet. Med. 23 (2006) 839–844.
[69] M.C. Thomas, M.E. Cooper, C. Tsalamandris, et al., Anemia with impaired erythropoietin response in diabetic patients, Arch. Intern. Med. 165 (2005) 466–469.
[70] K.J. Craig, J.D. Williams, S.G. Riley, et al., Anemia and diabetes in the absence of nephropathy, Diabetes Care 28 (2005) 1118–1123.
[71] S. Inomata, M. Itoh, H. Imai, T. Sato, Serum levels of erythropoietin as a novel marker reflecting the severity of diabetic nephropathy, Nephron 75 (1997) 426–430.
[72] A. Symeonidis, A. Kouraklis-Symeonidis, A. Psiroyiannis, et al., Inappropriately low erythropoietin response for the degree of anemia in patients with noninsulin-dependent diabetes mellitus, Ann. Hematol. 85 (2006) 79–85.
[73] M.C. Thomas, The high prevalence of anemia in diabetes is linked to functional erythropoietin deficiency, Semin. Nephrol. 26 (2006) 275–282.
[74] M.C. Thomas, W.C. Burns, M.E. Cooper, Tubular changes in early diabetic nephropathy, Adv. Chronic. Kidney Dis. 12 (2005) 177–186.
[75] M.C. Thomas, C. Tsalamandris, R. MacIsaac, et al., Low-molecular-weight AGEs are associated with GFR and anemia in patients with type 2 diabetes, Kidney Int. 66 (2004) 1167–1172.
[76] W. Jelkmann, C. Bauer, beta 2-Adrenergic stimulation of erythropoiesis in busulfan treated mice, Exp. Hematol. 8 (1980) 742–748.
[77] K. Obayashi, Y. Ando, H. Terazaki, et al., Mechanism of anemia associated with autonomic dysfunction in rats, Auton. Neurosci. 82 (2000) 123–129.
[78] J. Zivny, B. Ostadal, J. Neuwirt, et al., Effect of beta adrenergic blocking agents on erythropoiesis in rats, J. Pharmacol. Exp. Ther. 226 (1983) 222–225.
[79] B.L. Ebert, H.F. Bunn, Regulation of the erythropoietin gene, Blood 94 (1999) 1864–1877.
[80] G.L. Semenza, M.K. Nejfelt, S.M. Chi, S.E. Antonarakis, Hypoxia-inducible nuclear factors bind to an enhancer element located 3' to the human erythropoietin gene, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 5680–5684.
[81] G.L. Semenza, Hypoxia-inducible factor 1 (HIF-1) pathway, Sci. STKE 2007 (2007) cm8.
[82] G.L. Wang, B.H. Jiang, E.A. Rue, G.L. Semenza, Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 5510–5514.
[83] G.L. Semenza, HIF-1: mediator of physiological and pathophysiological responses to hypoxia, J. Appl. Physiol. 88 (2000) 1474–1480.
[84] G.L. Wang, G.L. Semenza, General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 4304–4308.
[85] H.F. Bunn, J. Gu, L.E. Huang, et al., Erythropoietin: a model system for studying oxygen-dependent gene regulation, J. Exp. Biol. 201 (1998) 1197–1201.
[86] G.L. Semenza, Hypoxia-inducible factor 1: master regulator of O2 homeostasis, Curr. Opin. Genet. Dev. 8 (1998) 588–594.
[87] E.B. Rankin, M.P. Biju, Q. Liu, et al., Hypoxia-inducible factor-2 (HIF-2) regulates hepatic erythropoietin in vivo, J. Clin. Invest. 117 (2007) 1068–1077.
[88] T. Tanaka, H. Kato, I. Kojima, et al., Hypoxia and expression of hypoxia-inducible factor in the aging kidney, J. Gerontol., A, Biol. Sci. Med. Sci. 61 (2006) 795–805.
[89] C. Di Giulio, G. Bianchi, M. Cacchio, et al., Oxygen and life span: chronic hypoxia as a model for studying HIF-1alpha, VEGF and NOS during aging, Respir. Physiol. Neurobiol. 147 (2005) 31–38.
[90] A. Rivard, L. Berthou-Soulie, N. Principe, et al., Age-dependent defect in vascular endothelial growth factor expression is associated with reduced hypoxia-inducible factor 1 activity, J. Biol. Chem. 275 (2000) 29643–29647.
[91] G. Frenkel-Denkberg, D. Gershon, A.P. Levy, The function of hypoxia-inducible factor 1 (HIF-1) is impaired in senescent mice, FEBS Lett. 462 (1999) 341–344.
[92] H.S. Chahal, W.M. Drake, The endocrine system and ageing, J. Pathol. 211 (2007) 173–180.
[93] A. Gray, H.A. Feldman, J.B. McKinlay, C. Longcope, Age, disease, and changing sex hormone levels in middle-aged men: results of the Massachusetts Male Aging Study, J. Clin. Endocrinol. Metab. 73 (1991) 1016–1025.
[94] J.E. Morley, F.E. Kaiser, H.M. Perry III, et al., Longitudinal changes in testosterone, luteinizing hormone, and follicle-stimulating hormone in healthy older men, Metabolism 46 (1997) 410–413.
[95] S.M. Harman, E.J. Metter, J.D. Tobin, et al., Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging, J. Clin. Endocrinol. Metab. 86 (2001) 724–731.
[96] S. Shaldon, K.M. Koch, F. Oppermann, et al., Testosterone therapy for anaemia in maintenance dialysis, Br. Med. J. 3 (1971) 212–215.
[97] B.D. Doane, W. Fried, F. Schwartz, Response of uremic patients to nandrolone decanoate, Arch. Intern. Med. 135 (1975) 972–975.
[98] D.C. Cattran, S.S. Fenton, D.R. Wilson, et al., A controlled trial of nondrolone decanoate in the treatment of uremic anemia, Kidney Int. 12 (1977) 430–437.
[99] R. Alexanian, Erythropoietin and erythropoiesis in anemic man following androgens, Blood 33 (1969) 564–572.
[100] J.C. Schooley, Inhibition of erythropoietic stimulation by testosterone in polycythemic mice receiving anti-erythropoietin, Proc. Soc. Exp. Biol. Med. 122 (1966) 402–403.
[101] J.L. Teruel, R. Marcen, J.F. Navarro, et al., Evolution of serum erythropoietin after androgen administration to hemodialysis patients: a prospective study, Nephron 70 (1995) 282–286.
[102] J.W. Byron, Effect of steroids on the cycling of haemopoietic stem cells, Nature 228 (1970) 1204.
[103] D.K. Ornstein, J.A. Beiser, G.L. Andriole, Anaemia in men receiving combined finasteride and flutamide therapy for advanced prostate cancer, BJU Int. 83 (1999) 43–46.
[104] J.P. Weber, P.C. Walsh, C.A. Peters, J.L. Spivak, Effect of reversible androgen deprivation on hemoglobin and serum immunoreactive erythropoietin in men, Am. J. Hematol. 36 (1991) 190–194.
[105] S.B. Strum, J.E. McDermed, M.C. Scholz, et al., Anaemia associated with androgen deprivation in patients with prostate cancer receiving combined hormone blockade, Br. J. Urol. 79 (1997) 933–941.
[106] L. Ferrucci, M. Maggio, S. Bandinelli, et al., Low testosterone levels and the risk of anemia in older men and women, Arch. Intern. Med. 166 (2006) 1380–1388.
[107] J.F. Tangredi, I.L. Buxton, Hypertension as a complication of topical testosterone therapy, Ann. Pharmacother. 35 (2001) 1205–1207.
[108] Physician's Package Insert, AndroGel [http://www.fda.gov/MedWatch/SAFETY/2004/nov_PI/AndroGel_PI.pdf].
[109] E. Corpas, S.M. Harman, M.R. Blackman, Human growth hormone and human aging, Endocr. Rev. 14 (1993) 20–39.
[110] J.D. Veldhuis, A. Iranmanesh, C.Y. Bowers, Joint mechanisms of impaired growth-hormone pulse renewal in aging men, J. Clin. Endocrinol. Metab. 90 (2005) 4177–4183.
[111] S. Merchav, I. Tatarsky, Z. Hochberg, Enhancement of erythropoiesis in vitro by human growth hormone is mediated by insulin-like growth factor I, Br. J. Haematol. 70 (1988) 267–271.
[112] G. Rodriguez-Tarduchy, M.K. Collins, I. Garcia, A. Lopez-Rivas, Insulin-like growth factor-I inhibits apoptosis in IL-3-dependent hemopoietic cells, J. Immunol. 149 (1992) 535–540.
[113] K. Muta, S.B. Krantz, Apoptosis of human erythroid colony-forming cells is decreased by stem cell factor and insulin-like growth factor I as well as erythropoietin, J. Cell. Physiol. 156 (1993) 264–271.

[114] S. Merchav, I. Silvian-Drachsler, I. Tatarsky, et al., Comparative studies of the erythroid-potentiating effects of biosynthetic human insulin-like growth factors-I and -II, J. Clin. Endocrinol. Metab. 74 (1992) 447–452.
[115] M. Sohmiya, Y. Kato, Human growth hormone and insulin-like growth factor-I inhibit erythropoietin secretion from the kidneys of adult rats, J. Endocrinol. 184 (2005) 199–207.
[116] G.J. Fruhman, R. Gerstner, A.S. Gordon, Effects of growth hormone upon erythropoiesis in the hypophysectomized rat, Proc. Soc. Exp. Biol. Med. 85 (1954) 93–96.
[117] S. Bergamaschi, C. Giavoli, E. Ferrante, et al., Growth hormone replacement therapy in growth hormone deficient children and adults: effects on hemochrome, J. Endocrinol. Invest. 29 (2006) 399–404.
[118] M. Sohmiya, Y. Kato, Effect of long-term administration of recombinant human growth hormone (rhGH) on plasma erythropoietin (EPO) and haemoglobin levels in anaemic patients with adult GH deficiency, Clin. Endocrinol. (Oxf) 55 (2001) 749–754.
[119] E.R. Christ, M.H. Cummings, N.B. Westwood, et al., The importance of growth hormone in the regulation of erythropoiesis, red cell mass, and plasma volume in adults with growth hormone deficiency, J. Clin. Endocrinol. Metab. 82 (1997) 2985–2990.
[120] T. Ganz, Molecular pathogenesis of anemia of chronic disease, Pediatr. Blood Cancer 46 (2006) 554–557.
[121] N.C. Andrews, Anemia of inflammation: the cytokine-hepcidin link, J. Clin. Invest. 113 (2004) 1251–1253.
[122] T. Ganz, Hepcidin-a regulator of intestinal iron absorption and iron recycling by macrophages, Best Pract. Res. Clin. Haematol. 18 (2005) 171–182.
[123] H. Bruunsgaard, K. Andersen-Ranberg, B. Jeune, et al., A high plasma concentration of TNF-alpha is associated with dementia in centenarians, J. Gerontol., A, Biol. Sci. Med. Sci. 54 (1999) M357–M364.
[124] G. Paolisso, M.R. Rizzo, G. Mazziotti, et al., Advancing age and insulin resistance: role of plasma tumor necrosis factor-alpha, Am. J. Physiol. 275 (1998) E294–E299.
[125] J. Wei, H. Xu, J.L. Davies, G.P. Hemmings, Increase of plasma IL-6 concentration with age in healthy subjects, Life Sci. 51 (1992) 1953–1956.
[126] M. Maggio, J.M. Guralnik, D.L. Longo, L. Ferrucci, Interleukin-6 in aging and chronic disease: a magnificent pathway, J. Gerontol., A, Biol. Sci. Med. Sci. 61 (2006) 575–584.
[127] L. Ferrucci, A. Corsi, F. Lauretani, et al., The origins of age-related proinflammatory state, Blood 105 (2005) 2294–2299.
[128] L. Ferrucci, J.M. Guralnik, R.C. Woodman, et al., Proinflammatory state and circulating erythropoietin in persons with and without anemia, Am. J. Med. 118 (2005) 1288.
[129] Y. Kamenetz, Y. Beloosesky, C. Zeltzer, et al., Relationship between routine hematological parameters, serum IL-3, IL-6 and erythropoietin and mild anemia and degree of function in the elderly, Aging (Milano) 10 (1998) 32–38.
[130] L. Ferrucci, J.M. Guralnik, S. Bandinelli, et al., Unexplained anaemia in older persons is characterised by low erythropoietin and low levels of pro-inflammatory markers, Br. J. Haematol. 136 (2007) 849–855.
[131] Y. Beguin, Soluble transferrin receptor for the evaluation of erythropoiesis and iron status, Clin. Chim. Acta 329 (2003) 9–22.