BIOCHEMICAL PHARMACOLOGY 77 (2009) 1572–1579

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Commentary

Linking anemia to inflammation and cancer: The crucial role of TNFα

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ARTICLE INFO

Keywords:
TNFα
Erythropoietin
Erythroid differentiation
Inflammation
Cancer

ABSTRACT

Erythropoiesis is considered as a multistep and tightly regulated process under the control of a series of cytokines including erythropoietin (Epo). Epo activates specific signaling pathways and leads to activation of key transcription factors such as GATA-1, in order to ensure erythroid differentiation. Deregulation leads to a decreased number of red blood cells, a hemoglobin deficiency, thus a limited oxygen-carrying capacity in the blood. Anemia represents a frequent complication in various diseases such as cancer or inflammatory diseases. It reduces both quality of life and prognosis in patients. Tumor necrosis factor alpha (TNFα) was described to be involved in the pathogenesis of inflammation and cancer related anemia. Blood transfusions and erythroid stimulating agents (ESAs) including human recombinant Epo (rhuEpo) are currently used as efficient treatments. Moreover, the recently described conflicting effects of ESAs in distinct studies require further investigations on the molecular mechanisms involved in TNFα-caused anemia. The present study aims to evaluate the current knowledge and the importance of the effect of the proinflammatory cytokine TNFα on erythropoiesis in inflammatory and malignant conditions.

1. Introduction

The proinflammatory cytokine tumor necrosis factor (TNFα) was brought in connection with inflammation and cancer, two tightly linked research areas [1–3] and it was demonstrated that cancer-associated inflammation could promote tumor growth [1,4,5]. TNFα expression has been confirmed in the tumor microenvironment of various malignancies [6] and was categorized as a tumor promoter because of its effects on tumor initiation and progression [7,8]. Furthermore there are more and more drugs in clinical development that modulate TNFα function in a wide range of inflammatory diseases and cancers [8].

Cancer and inflammation related anemia were shown to be mediated by cytokine release, and particularly by TNFα, interferon-β (IFN-β), and interleukin-1 (IL-1) [9]. Moreover, inhibition of colony-forming units-erythroid (CFU-E) in uremic patients with inflammatory disease due to TNFα and IFN-γ

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Abbreviations: Epo, erythropoietin; NF-κB, nuclear factor-kappa B; FOG-1, friend of GATA; NF-E2, nuclear factor erythroid 2; EKLF, erythroid krüppel like factor; SCF, stem cell factor; EpoR, epo receptor; HIF, hypoxia inducible factor; HSC, hematopoietic stem cell factor; BFU-E, burst-forming unit-erythroid; CFU-E, colony-forming unit-erythroid; FA, fanconi anemia; GPA, glycophorin A; TNFα, tumor necrosis factor alpha; TNFR, TNFα receptor; ESA, erythroid stimulating agent.

0006-2952/$ – see front matter
doi:10.1016/j.bcp.2008.12.018

release has been reported [10]. Following anti-TNFα therapy, patients with rheumatoid arthritis showed improvement in anemic symptoms [11]. Since several years, human recombinant erythropoietin (rhuEpo) is a specific remedy administered against cancer-associated anemia. This treatment has a positive impact on hemoglobin levels and patient quality of life is improved. However, a preclinical background and some clinical data suggest a detrimental role of Epo in cancer by a possible stimulation of tumor growth.

2. Regulation of erythropoiesis

Hematopoiesis is the physiological process that leads to the formation of circulating blood cells from common hematopoietic stem cells (HSCs) in the bone marrow. The different mature hematopoietic cells are usually classified in lymphoid and myeloid lineages. They are regulated by distinct cytokines acting on multipotential progenitors and their committed offspring [12,13] (Fig. 1).

Erythropoiesis is a multistep event leading to the formation of erythrocytes. Erythroid differentiation arises from the myeloid root and is phenotypically characterized by the production of hemoglobin and expression of erythroid markers (Fig. 2). During differentiation from a multipotent common myeloid progenitor (CMP) to a bipotent megakaryocytic/erythroid progenitor (MEP), burst-forming units-erythroid (BFU-E) and CFU-E are the earliest identifiable erythroid progenitors in culture (Fig. 1). BFU-E and CFU-E are characterized by their *in vitro* ability to form colonies.

Erythropoiesis is a very dynamic and tightly regulated process by which 2×10^{11} erythrocytes (lifespan of 100–120 days) are produced every day. Ferrous iron (Fe^{2+}) is essential for erythropoiesis as a major component of heme in hemoglobin as well as in the redox system of the respiratory chain. Hepcidin, a 25-amino acid peptide, is the main regulator of iron transport. A feedback loop involving the major cytokine for human erythropoiesis, Epo, regulates this physiological process, but other cytokines and nuclear hormones are also involved. IL-3 increases the number of BFU-E, whereas stem

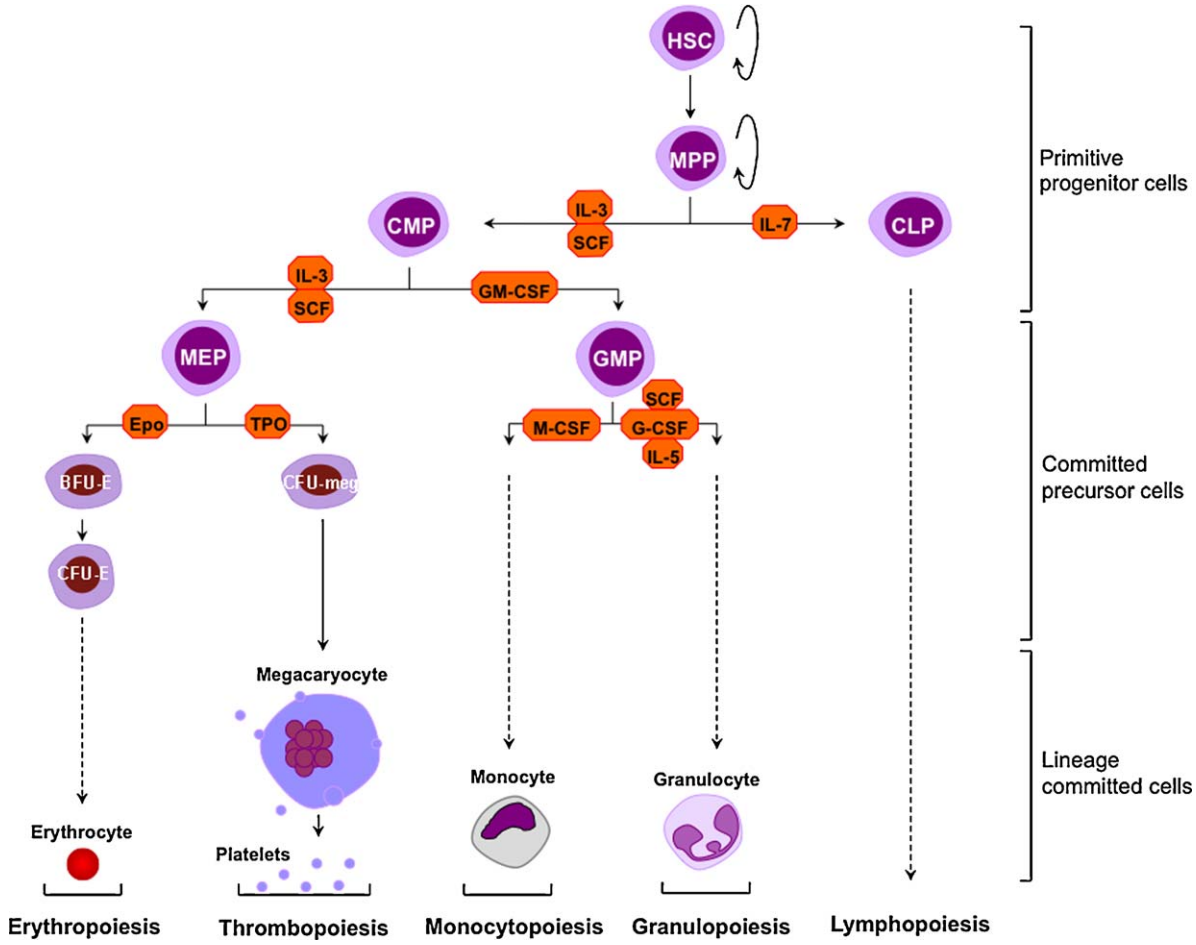

Fig. 1 – Hematopoiesis and the role of cytokines. Cytokines act both on multipotential progenitors and their committed offspring. CLP, common lymphoid progenitor; CMP, common myeloid progenitor; HSC, hematopoietic stem cell; GMP, granulocyte–macrophage progenitor; MEP, megakaryocyte erythroid progenitor; BFU-E, burst-forming units-erythroid; CFU-E, colony-forming units-erythroid; CFU-Meg, colony-forming units-megakaryocyte; MPP, multipotent progenitor; IL, interleukin; SCF, stem cell factor; GM-CSF, granulocyte–macrophage colony stimulating factor; G-CSF, granulocyte colony stimulating factor; M-CSF, macrophage colony stimulating factor; TPO, thrombopoietin; Epo, erythropoietin (adapted from Refs. [12,13] with modifications).

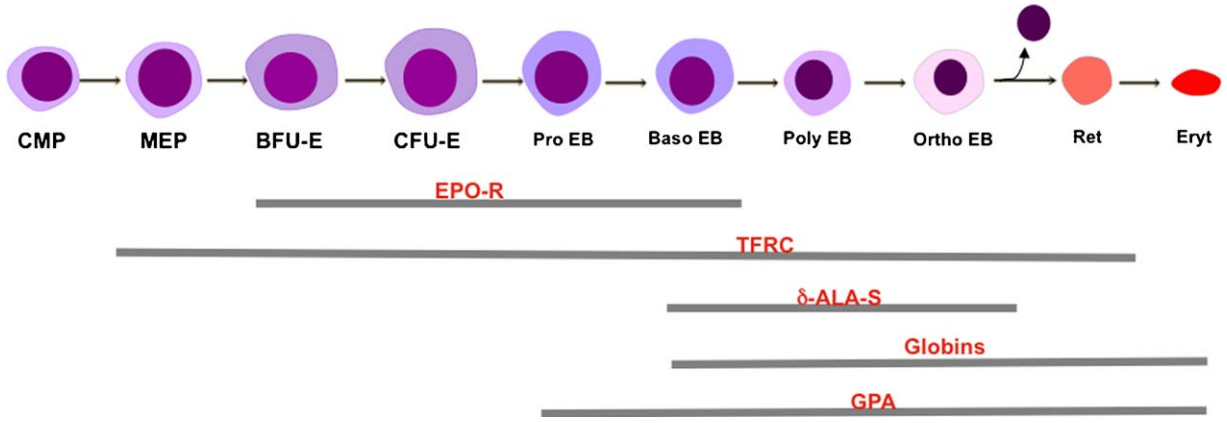

Fig. 2 – Stages of mammalian erythropoiesis and corresponding expression of erythroid specific markers. The relative sizes and morphologic appearances of erythroid cells at various stages of differentiation: common myeloid progenitor (CMP), megakaryocyte erythroid progenitor (MEP), burst-forming units-erythroid (BFU-E), colony-forming units-erythroid (CFU-E), proerythroblasts (Pro EB), basophilic erythroblasts (Baso EB), polychromatophilic erythroblasts (Poly EB), orthochromatic erythroblasts (Ortho EB), reticulocytes (RET), and erythrocytes (Eryt). Erythroid markers are represented in red and their periods of expression with gray lines: erythropoietin receptor (EpoR), glycophorin A (GPA), transferrin receptors (TFRCs), δ-aminolevulinate synthase (δ-ALA-S) (adapted from Ref. [81]).

cell factor (SCF) raises the number of cells within BFU-E and CFU-E (Fig. 1).

Kidney and liver are the main sites that produce the Epo in adult humans. The rate of Epo gene expression depends on the level of tissue oxygen through the availability of the hypoxia inducible factor (HIF). HIF heterodimer is composed of the oxygen sensitive HIF-1α and the constitutively expressed HIF-1β subunits. In hypoxic conditions HIF interacts with specific binding sites in the Epo enhancer. Oxygen-dependent prolyl hydroxylases control Epo variations in the kidney by regulating the stability of HIF-1α. The number of circulating erythrocytes is directly dependent on Epo amount in blood.

Epo is implicated in the control of cell survival, proliferation and differentiation within the erythroid pathway. It acts through its receptor (EpoR) in order to stimulate underlying cell signaling pathways including the Phosphatidylinositol 3 kinase (PI3K), the Janus kinase (JNK)/signal-transducer and activator of transcription (STAT) and the mitogen-activated protein kinase (MAPK)/extracellular signal-related kinase (ERK) pathways [14]. Moreover, Epo has been reported to modulate GATA-1 function in erythroid cells [15].

The survival of erythroid precursors and their terminal differentiation into red blood cells depends on Epo/EpoR interaction and GATA-1 transcription factor activity. GATA-1 was first identified as a protein with binding capacity to the β-globin promoter [16]. It is a member of the GATA family, which includes 6 members (GATA-1 to GATA-6). These transcription factors recognize the same DNA consensus sequence (A/T)GATA(A/G) and present two characteristic zinc finger motifs specific to the GATA family [17]. Three functional domains compose GATA-1 protein: the N-terminal Zinc finger that supplies the stabilization and specificity of DNA-binding and is responsible for the interactions with cofactors, the C-terminal Zinc finger that is essential for binding to the GATA consensus sequence of the DNA and the N-terminal activation domain [18].

GATA-1 is required for terminal erythroid maturation [19]. Indeed, its crucial role in erythropoiesis was shown using GATA-1 null mouse embryos, which died between E10.5 and E12.5 from severe anemia due to a complete ablation of embryonic erythropoiesis [20]. Moreover, GATA-1−/− embryonic stem cells cannot contribute to definitive erythropoiesis [21].

GATA-1 activity is dependent on protein–protein interactions, involving cofactors with either promoting or repressing activities [18] (Table 1). The transcription factor PU.1, an Ets family member of transcription factors, is required for the development of the myeloid and lymphoid lineages. Nevertheless, its inhibitory effect on GATA-1 activity can prevent erythroid differentiation. PU.1 and GATA-1 have a cross-antagonistic relationship. Indeed, GATA-1 and PU.1 seem to functionally antagonize each other via direct physical interaction of their DNA-binding domains. PU.1 impairs GATA-1 by inhibiting its binding to DNA while GATA-1 inhibits PU.1 by preventing its interaction with c-Jun [22].

Table 1 – The main transcription factors and cofactors involved in the regulation of erythroid specific genes expression and their positive (+) or negative (−) effect on erythropoiesis.

Factor name	Effect	References
GATA-1	+	[20]
GATA-2	+/−	[82]
NF-E2 (nuclear factor erythroid 2)	+	[83]
FOG (friend of GATA)-1	+	[84]
Lmo2 (LIM-only protein 2) (Rbtn2)	+	[85]
p300/CBP	+	[86]
EKLF (erythroid kruppel like factor)	+	[87]
PU.1 (SPI1)	−	[22]
c-Myb	−	[88]

In summary, GATA-1 activity is dependent on complex positive and negative interactions with transcriptional cofactors as well as posttranslational modifications leading to modulation of erythroid lineage-specific genes transcription.

GATA-2, nuclear factor erythroid-2 (NF-E2) and erythroid Krüppel-like factor (EKLF) are other specific transcription factors that have a major influence on erythroid differentiation and that are activated during erythropoiesis. GATA-2 is overexpressed during early hematopoiesis resulting in maintenance of the renewal capacity of erythroid progenitor cells. Its expression is then progressively repressed by the increasing expression of GATA-1 [23]. NF-E2 is described to act as a major regulator of hemoglobin synthesis during erythropoiesis [24], and EKLF, as a crucial factor in erythroid and megakaryocytic differentiation and maturation [25,26] (Table 1).

Deregulation of Epo or other key factors of erythroid differentiation can lead to major changes in red blood cell number, and subsequent decrease in the oxygen-carrying capacity of the blood. Erythrocytosis are disorders resulting in an excessively high level of erythrocytes, whereas anemia is characterized by a qualitative or quantitative deficiency of hemoglobin. Anemia is clinically defined by a hemoglobin (Hb) level inferior to 12 g/dL.

3. Tumor necrosis factor alpha

TNFα also known as cachectin or differentiation inducing factor (DIF), is a proinflammatory multifunctional cytokine, which is mainly produced by macrophages, but also by neutrophiles, fibroblasts, keratinocytes, astrocytes, Kupffer cells, smooth-muscle cells, T and B cells. It was initially described to induce hemorrhagic necrosis in transplanted tumors [27]. TNFα effects are principally mediated through two distinct receptors TNFα receptor (TNFR) I and II. TNFα/TNFR interaction results in stimulation of the underlying cell signaling pathways that lead to nuclear factor kappa B (NF-κB), c-Jun N-terminal kinase (JNK), p38MAPK, or caspase activation. Thus TNFα simultaneously activates both apoptotic and anti-apoptotic or cell survival signals depending on the factors present in the receptor complex [28]. TNFα, which was discovered in B cells, is known as the most powerful activator of NF-κB transcription factor. NF-κB is known to bind its specific ten base pair consensus-binding site in order to regulate over 200 immune, growth, and inflammation genes.

TNFα, as well as other TNFα superfamily members, plays a role in hematopoiesis, host defense, immune surveillance, and proliferation. In this regard, TNFα deregulation leads to numerous diseases, including cancer [28–30].

4. TNFα and inflammation

A TNFα overproduction is involved in numerous chronic inflammatory diseases, such as rheumatoid arthritis [31] chronic hepatitis C [32], or Crohn's disease [33]. An increase in the TNFα level was described in diabetic patients to cause retinopathies [34], while during pancreatitis, the release of TNFα leads to inflammation and cellular damage [35].

Currently, three marketed TNFα antagonists [etanercept (Enbrel®), infliximab (Remicade®), and adalimumab (Humira®)] are indicated in diseases characterized by abnormally elevated TNFα levels. Moreover the effectiveness of the treatments varies with agent and disease [36,37]. TNFα is thus leading to various biological phenomena implying different molecular mechanisms and is involved in different cellular responses. Although TNFα is considered to act as a proinflammatory cytokine, it was described as a positive and negative regulator of myeloid cell proliferation and differentiation [38–40]. Effects of TNFα can be mediated either directly [41] or indirectly by inducing other cells to produce cytokines, including hematopoietic growth factors [42,43].

5. Link between TNFα, inflammation and cancer

Abnormal TNFα levels have been confirmed in tumor microenvironment [6]. Moreover, this cytokine is paradoxically able to induce necrosis and to promote tumor development, depending on the levels of TNFα in distinct settings [44]. When TNFα is secreted by tumors and tumor-associated macrophages, it promotes tumor growth and stimulates angiogenesis, whereas when it is administered therapeutically at high doses, it induces an increased permeability of tumor vasculature. Thus, recombinant TNFα, as a tumor regressing agent, is approved in Europe to be administered locoregionally at supraphysiological levels as a therapy for soft tissue sarcoma [45].

Using murine models, it was shown that inflammation-associated hepatocellular carcinogenesis involved the activation of the tumor promoter NF-κB via the production of TNFα [5]. Moreover several reports associate detection of abnormally high levels of TNFα protein and/or constitutively active NF-κB in cancer patients with a wide range of tumor types [46], including kidney [47], breast [48], asbestosis induced lung [49], and prostate cancers [50]. Suppression of constitutively active NF-κB results in cell proliferation arrest and apoptosis, indicating a crucial role for NF-κB in proliferation and survival [51]. Furthermore chronic bioavailability of TNFα has been correlated with enhanced invasive activities as well as survival of neoplastic cells [44]. Within groups of patients with the same tumor type, higher levels of TNFα have been correlated with advanced tumor stage, greater complications, and shorter survival time [52]. Moreover various cytokines, including TNFα, are overexpressed in pancreatic cancer cells, leading to an NF-κB activation and as a consequence, to cell growth by inhibiting apoptosis [53]. TNFα also appears as a growth factor regulated by NF-κB in Hodgkin's lymphoma, T cell lymphoma and glioma [54].

As Rudolf Virchow already suspected in 1863, inflammation and cancer have to be handled together [1,3]. Cancer-associated inflammation includes the expression of cytokines such as TNFα or IL-1 by tumor-associated macrophages, stimulating tumor growth [55]. As TNFα was categorized as a tumor promoter, it is not astonishing that cytokine antagonists as well as NF-κB inhibitors are already used in cancer therapy and prevention [7,8,44].

6. Deregulation of erythropoiesis by TNFα in inflammation and cancer

Besides the proinflammatory, proliferative and apoptotic properties, TNFα was also described as an inhibitor of the erythroid differentiation in *vitro* and in *vivo* [38,39,41,56] (Fig. 3). Its expression is associated with the hematologic diseases Fanconi anemia (FA) [57], myelodysplastic syndromes [58], aplastic anemia [59] and anemia due to chronic diseases [60]. Indeed, in FA patients, TNFα is significantly overexpressed in stimulated marrow mononuclear cells, which leads to a suppression of erythropoiesis. In bone marrow cultures, the addition of anti-TNFα increases the size and the number of CFU-E and BFU-E grown from FA patients but not from healthy controls. This indicates that FA subjects have a marrow TNFα activity that inhibits erythropoiesis *in vitro*. TNFα plays a relevant role in the pathogenesis of erythroid failure in FA patients [57]. Several *in vitro* studies revealed the inhibitory effects of this cytokine on hematopoietic progenitor cell growth [38,39,41,56,57,61]. It was shown that the inhibition of human CFU-E by TNFα requires IFN-β, which is produced by macrophages in response to TNFα. TNFα was also shown to have a direct inhibitory effect on Epo-induced generation of GPA positive cells from CD34+ progenitors, leading to the suppression of erythropoiesis and the reduction of the proliferation capacity of GPA positive cells [61]. Interestingly, TNFα is also believed to play a critical role in many forms of cancer [9,62] and inflammation related anemia [11]. Indeed anemia is considered as a common symptom induced by inflammation and cancer pathologies. In patients with B-cell chronic lymphocytic leukemia suffering from anemia, the serum levels of TNFα were significantly higher than in those without anemia [63]. The incidence of anemia was shown to vary with tumor type, stage and patient age. Up to one-third of patients had anemia at diagnosis [64]. This number increases after chemotherapy [65]. Cancer-associated anemia was shown to reduce survival of patients regardless of tumor type [66]. Moreover, the quality of life is considerably affected and is associated with a range of symptoms including fatigue, depression, and dizziness [67]; thus proinflammatory cytokines were recently suggested as the common denominator for cancer related fatigue [68]. Inflammation associated anemia is considered as a main symptom of patients with inflammatory disorders [69]. Prior to the use of erythroid

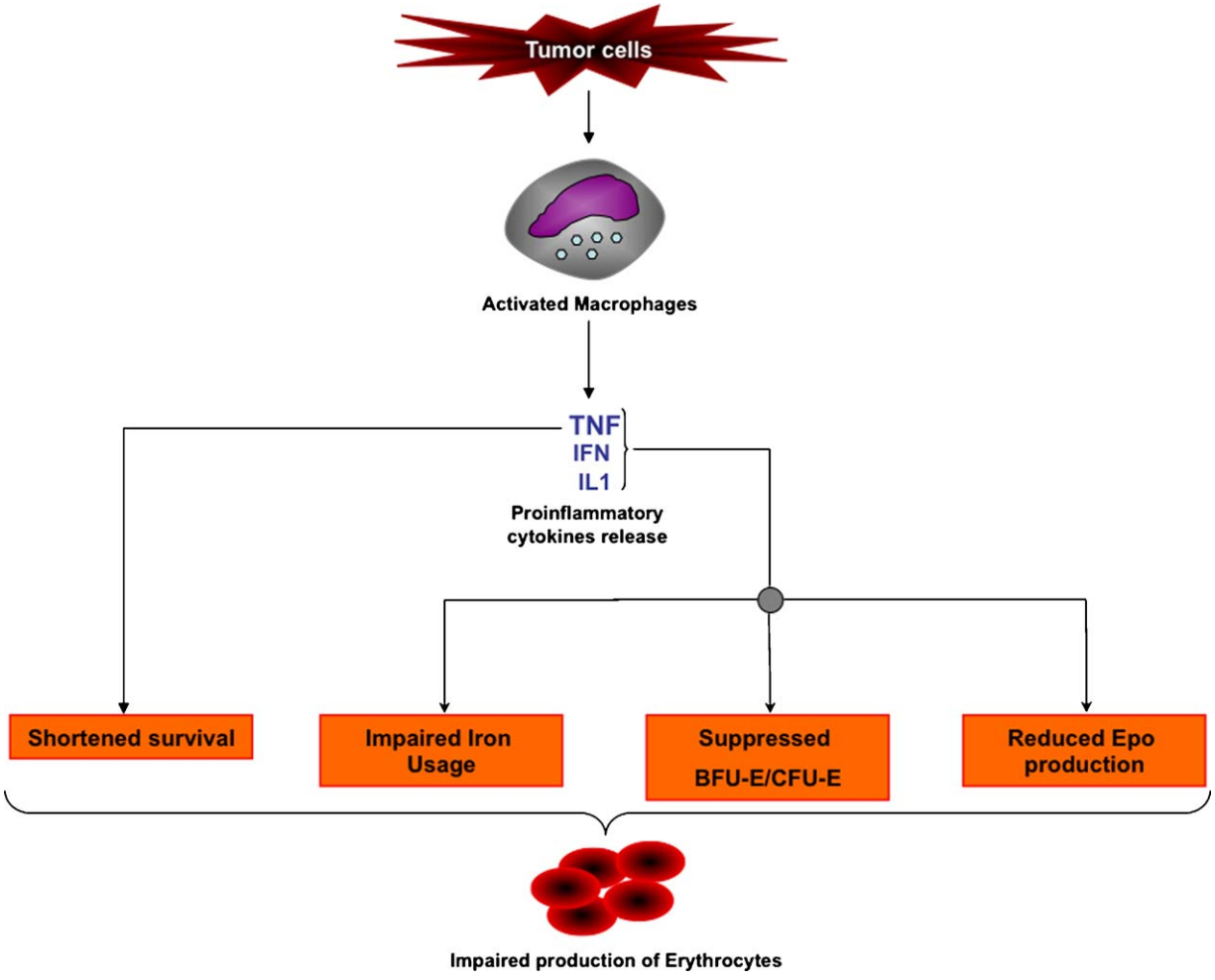

Fig. 3 – Pathophysiology of anemia. Tumor cells act on erythrocytes through macrophages by cytokine release, which leads to impaired erythropoiesis. Released cytokines can affect BFU-E and CFU-E proliferation, iron utilization and Epo production. TNFα can also affect erythrocyte half-life. TNFα, tumor necrosis factor alpha; IFN, interferon; IL, interleukin; BFU-E, burst-forming units-erythroid; CFU-E, colony-forming units-erythroid; Epo, erythropoietin (adapted from Ref. [68]).

stimulating agents (ESAs), the most frequent treatment of cancer related anemia was blood transfusion. Clinical trials established erythropoietin's ability to increase hemoglobin levels and reduce transfusion requirements [2,3]. However, the conflicting effects of ESAs were recently described in distinct studies [70–72]. Indeed, besides cardiovascular and thromboembolic events in erythropoietin-treated patients, several phase II and III trials showed a significant deterioration of cancer patients survival [73–75]. Unexpectedly, the increased mortality came from accelerated progression of cancer. This reproducible effect was attributed to erythropoietin. Thus, the use of erythropoietin in cancer patients might increase the risk of cancer-associated death. It is suggested that additional phase III trials should be performed to determine whether erythropoietin is safe when used in accordance with FDA-approved indications. One claims that waiting for these further studies to modify or stop Epo treatments could have detrimental impact on many cancer patients [70]. In this context, controversial effects of erythropoietin in cancer-related anemia makes necessary to further investigate the molecular mechanisms involved in anemia and to identify new targets for drug development as well as to detect more significant predictors. In order to improve quality of life, several drugs are under investigation for the treatment of different forms of anemia. Jelkmann reviewed several anti-anemic drugs and techniques based on Epo gene expression [71,76].

Additionally, our group previously reported that TNFα-mediated inhibition of K562 cell differentiation was correlated to GATA-1 downregulation [77], GATA-1/GATA-2 unbalance in favor of GATA-2 as well as a decrease in the acetylation status of GATA-1 [78,79]. Moreover we suggested a role for p38 in the inhibition of erythroid differentiation by TNFα, in correlation with a reversal of important erythroid transcription factors [78]. Miwatashi et al. already used a novel p38 inhibitor, N-[4-[2-ethyl-4-(3-methylphenyl)-1,3-thiazol-5-yl]-2-pyridyl]benzamide (TAK-715), as an anti-TNFα drug for the treatment of rheumatoid arthritis, presenting anemic complications [11,80].

7. Conclusions

Erythropoiesis is a tightly regulated, complex physiological process leading to the formation of erythrocytes from a pluripotent hematopoietic stem cell. Deregulation can lead to various complications, including anemia. Anemia represents a frequent complication in cancer patients, as well as in patients suffering from inflammatory diseases. Proinflammatory cytokines seem to be overexpressed in these diseases. Anemia considerably affects quality of life and is even considered as an independent bad prognostic factor. On the other hand some studies showed that the use of recombinant Epo as a treatment for cancer related anemia could be inappropriate for cancer patients. For these reasons the molecular mechanisms behind the inhibitory effect of TNFα on erythroid differentiation need to be further elucidated in order to find potential new, and more pointed therapeutic targets for inflammation and cancer related anemia. In this respect, investigations using hematopoietic stem cell culture systems should allow to better understand the impact of TNFα on the control of erythropoiesis by identifying which specific cellular process is affected, including differentiation and/or apoptosis regulation.

Acknowledgements

Research in M.D.'s lab is supported by the 'Recherche Cancer et Sang' foundation, the 'Recherches Scientifiques Luxembourg' association, Télévie and Action Lions Vaincre le Cancer. I.B. is supported by 'Action Lions Vaincre le Cancer' and C.G. by an AFR grant from FNR Luxembourg. The authors thank M. Groos for collaboration and 'Een Häerz fir kriibskrank Kanner' association for continuous support.

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