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Functional Links Between Glucocorticoids and Cytokines In DBA

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Abstract

Diamond Blackfan anaemia (DBA) is a red blood cell aplasia characterized by erythropoietic defects as well as congenital anomalies. Forty percent of patients with DBA are treated with glucocorticoid steroids, which remain the mainstay of treatment in DBA. Many advances in the understanding of the physiological role of the glucocorticoid receptor have been made since the first introduction of glucocorticoids to the clinic, but their mechanism of action in the treatment of DBA is still under investigation. This review is intended to summarize the mechanism of glucocorticoid action specifically as related to erythropoiesis, focusing on the functional links between glucocorticoids and cytokines.

Introduction

Diamond Blackfan anemia (DBA) was first described as a disorder of impaired red blood cell production in children [1,2]. While most of the DBA cases are diagnosed in early infancy, a recent case report reveals that DBA can occur during fetal development. This suggests that severely affected DBA fetus likely die to hydrops fetalis and result in undiagnosed miscarriages [3]. This disorder results from a cellular defect in which erythroid progenitors are highly sensitive to death by apoptosis, leading to erythropoietic failure [4]. The etiology of DBA has been the subject of continuous discussions, and while the early success of treatment with glucocorticoids (GCs) in 1951 [5] led to the idea that the pathogenesis of DBA could have an immunologic basis [6], it is now accepted that DBA is in fact a member of a rare group of genetic disorders.

Approximately 50% of DBA patients have a single mutation in a gene encoding a ribosomal protein, which indicates that DBA is associated with a disorder of ribosome biogenesis and/or function [4]. The pronounced erythroid defect suggests that erythroid progenitors may express specific mRNAs that are hypersensitive to the decreased translation capacity [7]. Twenty-five percent of patients have a mutation in the ribosomal protein S19 (RPS19) gene [8], and two independent studies have demonstrated that over expression of the RPS19 transgene increases the number of erythroid colonies in RPS19 deficient hematopoietic progenitor cells in vitro [9,10]. In a mouse model of DBA, a high expression of RPS19 can rescue the erythroid development, and the corrected DBA cells show a survival advantage in vivo [11]. As zebrafish hematopoietic regulation is conserved with mammals, zebrafish models have also been reported to be useful in studying DBA [12-14], recapitulating many aspects of the DBA phenotype, including hematopoietic specific defects and p53 activation [14]. The list of genes that are mutated in DBA has been updated in 2013, to include ten ribosomal genes and one transcriptional regulator: RPS19, RPS24, RPS17, RPL35A, RPL5, RPL11, RPS7, RPS10, RPS26, RPL26, and GATA1 [15].

More than 50 years after their introduction to the clinic, GCs are still the most effective drugs used in DBA. Nevertheless, the reported side effects of GCs include decreased growth velocity in infants, neuromotor dysfunction in toddlers, and significant immune suppression, rendering GCs an unsuitable therapeutic choice for children under the age of one [4]. Today, the only curative treatment for DBA is hematopoietic stem cell transplantation, a treatment that relies on the availability of an HLA-matched related donor [4]. To reduce the side effects of GCs and increase their efficacy, the mechanism of action of GCs has been – and still is – the subject of intensive research.

This short review will therefore focus on (a) the molecular mechanisms of GCs reported in erythropoiesis, and (b) the relationship between GCs and cytokines, where cytokines will be discussed as new potential drug targets in DBA treatment.

Molecular mechanism of Glucocorticoids

Glucocorticoids (GCs) have pleiotropic effects on hematopoietic cells. They have been shown to induce apoptosis in lymphoid cells [16], prevent apoptosis in granulocytes [17], and drive the proliferation of erythroid progenitors [18,19].

Endogenous GCs

GCs play a pivotal role in several critical biologic processes including growth, reproduction, intermediary metabolism, immune and inflammatory reactions and they also have specific functions in the central nervous and cardiovascular systems [20,21]. The principal endogenous GCs are the hormones cortisol and corticosterone. Cortisol and corticosterone are both synthesized from cholesterol in cells of the zona fasciculate of the adrenal cortex (cortisol is the predominant GC in human, whereas rodents produce mainly corticosterone). The release of both GCs into the blood is pulsatile and varies according to a distinct circadian pattern. Additional secretions can occur independent of circadian tone in response to physical and/or emotional trauma [22].

In vitro, GCs enhance the formation of murine erythroid colonies [23] and increase proliferation of erythroid cells in the presence of limiting amounts of erythropoietin (Epo) [24]. Interestingly, GCs also stimulate erythropoiesis indirectly by increasing Epo production in the kidney [25].

Other studies have shown a direct correlation between GCs concentration and erythropoiesis by studying individuals that have pathological changes in the production of GCs. For example,

Addison's disease, a disorder caused by insufficient corticosteroid production, is associated with anemia, whereas increased red blood cell count, hemoglobin, and hematocrit values are observed in Cushing's syndrome, a disease characterized by elevated GCs levels [26].

The human glucocorticoid receptors

The glucocorticoid receptor (GR) is an intracellular receptor. It is the first member of the subfamily of steroid hormone receptors, is widely distributed in the body, and has a variety of functions that are essential for survival. Genetically modified mice lacking the GR die at birth and reveal impaired development of several organs [27]. Cell responsiveness to GCs depends not only on the presence of the GR but also on its concentration – which is known to fluctuate during development and the cell cycle, and following disturbances in endocrine status [28]. The human GR (hGR) gene consists of nine exons and is located on chromosome 5. Alternative splicing of the hGR gene in exon 9 generates two highly homologous isoforms of the receptor, termed α and β [29]. The GRα binds and is activated by corticosteroids, whereas the unique structure of the GRβ impairs the ligand-binding domain and induces nuclear retention [30]. The GRβ has been implicated in steroid resistance in asthma [31], and observed to exert dominant-negative effects on the GRα [32,33]. The belief that the GRβ is a general negative modulator of GRα function was challenged by cotransfection studies in COS-7 cells, where the GRβ was not observed to inhibit the effects of dexamethasone-activated GRα on a glucocorticoid-responsive reporter gene [34]. Most actions of GCs are mediated by the GR, and several polymorphisms in the hGR gene are associated with altered GC sensitivity [35]. As there is currently no reliable way to predict steroid responsiveness in DBA patients [4], these polymorphisms very likely affect the response of patients to GC in the treatment of DBA and represent another reason for the development of non GC-based DBA drugs.

Activation and targets of glucocorticoid receptor

Activation of the glucocorticoid receptor (GR) leads to subsequent intracellular signaling. Heat Shock Protein 90 (HSP90) regulates ligand binding and cytoplasmic retention of the GRα by exposing the ligand-binding site and masking the two nuclear localization sequences [36]. Upon ligand-induced activation, the GR undergoes a conformational change, resulting in its dissociation from a multi-protein complex that includes HSP90 and its translocation into the nucleus.

Within the nucleus, the GR binds as a homodimer to Glucocorticoid Response Elements (GREs) in the promoter region of target genes, regulating their expression positively or negatively depending on the GRE sequence and promoter context [37]. Alternatively, the ligand-activated GRα can interact as a monomer with other transcription factors, modulating their transcriptional activity through direct protein-protein interaction, independent of direct DNA binding [38].

DNA-independent actions of GR: transrepression

The suppression of transactivation of other transcription factors through protein-protein interactions is particularly important in the suppression of immune function and inflammation by GCs [39]. Most of the effects of GCs on the immune system may be mediated by the interaction between GR and NF-κB, AP-1, and signal transducers and activators of transcription (STATs) [40-43].

The example of transrepression of the proinflammatory transcription factor AP-1 is particularly interesting since DNA-binding inactive mutants of the GR are fully capable of AP-1 transrepression [44]. In erythroid progenitors, several members of NF-κB family are also expressed and it has been suggested that NF-κB factors could function to regulate specific genes involved in erythropoiesis. [45]. However, since induction of proliferation of erythroid progenitors by GCs requires DNA binding by the GR [18,46], the transrepression action has appeared not to be essential to the function of the GR in erythroid progenitors.

Transcriptional activation by GR: transactivation

DNA-dependent transactivation by the GR requires dimerization of the receptor [21,46]. Mice carrying a point mutation in the GR, which prevents receptor dimerization (GRdim/dim), are viable [46] and GRdim/dim mice display normal erythrocyte numbers in peripheral blood [47]. GRdim/dim mice were then challenged either by drug-induced hemolytic anemia or by hypoxia to address the question of whether or not the GR would be important for stress erythropoiesis. In both cases, wild-type mice responded with strongly increased numbers of colony-forming units–erythroid (CFU-Es) in their spleens, whereas GRdim/dim mice showed no response. GR-dependent expansion upon stress was restricted to a specific erythroid compartment [47]. In vitro, erythroid cells from fetal livers GRdim/dim mice fail to undergo sustained proliferation in contrast to wild-type cells [47].

Genes differentially regulated by GCs

Upon hormone binding, the GR associates with high affinity to genomic GR binding sequence, typically an imperfect palindromic hexameric half site, separated by 3-base pair spacers [48,49]. Genome-wide in silico identification of GR binding sequences could thus be a powerful method to reveal the transcriptional regulatory network of GCs. However, focusing on the role of the GC dexamethasone (Dex) in erythropoiesis, the Dex-induced genes are not enriched for GR binding sites in their promoter regions but instead for hypoxia-inducible factor 1α (HIF1α), suggesting that HIF1α activation would enhance the biologic function of GR activation [50]. Another oligonucleotide microarray study to survey gene expression in RPS19-deficient CD34+ cells showed that Dex decreases the expression of 3 cytokine genes: interleukin-1 beta (IL-1B), lymphotoxin-beta (LTB), and macrophage inflammatory protein-1-alpha (MIP-1α, CC chemokine ligand 3 [CCL3]) [51]. All of these cytokines are active in myeloid lineage cells, and Mip-1α is a cytokine that blocks proliferation of hematopoietic stem cells [52].

Targets of the GR include genes involved in proliferation of erythroblasts such as the SCF receptor Kit and the transcription factor Myb [18,53], and cytokines have been shown to modulate GCs function. In absence of the cytokines Epo and SCF, activation of the GR in erythroid progenitors causes cell cycle arrest [18,54]. Dex treatment leads to up-regulation of the antiproliferative genes Btg1 and GilZ [55]. In 3T3 fibroblasts, Btg1 expression is maximal in the G0/G1 phases of the cell cycle and is down-regulated when cells progress throughout G1, suggesting the existence of a functional link between Btg1 and cell cycle progression [56]. GilZ is known to interfere with signaling-controlled activity of NF-κB, AP-1 and the tyrosine kinase JAK2 inihibitor CIS [57,58]. In erythroid progenitors, GilZ is up-regulated by Dex alone, down-regulated by Epo and Epo/SCF, while coregulation by Epo, SCF, and Dex abolishes Epo-

dependent down-regulation [55]. These conclusions show that Dex is able to attenuate the effect of Epo and/or SCF. Furthermore, CXC chemokine receptor 4 (CXCR4) is up-regulated in the presence of Dex plus Epo/SCF, and CXCR4 is down regulated in GRdim/dim when compared with wt erythroid progenitors, thus confirming an activation of CXCR4 expression by GCs [55].

Suppressor of cytokine signaling 1 (SOCS1) up-regulation by Dex in erythroid progenitors has been confirmed by three different groups [50,55,59]. Mice lacking SOCS1 have been shown to have a subnormal hematocrit and an accumulation of immature red blood cells [60]. Furthermore, SOCS1 is associated with the phosphorylated form of JAK2 (pJAK2) [61] and dysregulation of pJAK2 degradation is involved in polycythemia [62]. Polycythemia is characterized by a net increase in the total number of red blood cells, and can arise when an intrinsic defect in erythroid progenitors render them hypersensitive or independent of erythropoietin stimulation [63]. These reports suggest that SOCS1 might be one of the GCs targets in DBA.

Other mechanisms of action of GCs

The effects of GCs on inflammation occur in a time-frame of 30 minutes to 18 hours [64]. However, the previously described mechanisms cannot explain the rapid effects observed within minutes or seconds following administration. As reported in several other cellular systems [65], the GR can activate a rapid membrane-associated signaling in erythroid cells. In human erythroblasts, growth factors stimulation induces association of the GR on the membrane, where the GR forms a complex with the EPO receptor to antagonize its ability to phosphorylate STAT5 [66]. This reveals that GCs are able to inhibit erythroid maturation through a rapid membrane-associated pathway that interferes with EPO receptor signaling.

Another mechanism is based on a direct interaction of the GR with mRNA. Whereas some RNA-binding proteins (RBPs) such as ubiquitous HuR mainly act as a positive regulator of mRNA stability, others such as the GCs-induced zinc finger RBP tristetraprolin (TTP) limit the inflammatory response by accelerating the mRNA decay of its targets [67]. Interacting directly with the chemokines (C-C motif) ligand 2 and 7 (CCL2 and CCL7) mRNAs in human airway epithelial cell line BEAS-2B, the GR accelerates the decay of their respective mRNA [68]. The identification of GR-mediated acceleration of chemokine mRNA decay and of this additional function of GR in the cytoplasmic compartment opens a new paradigm in the GCs mechanism of action. Human CD34+ cells and hematopoietic precursors secrete numerous regulatory molecules that form the basis of intercellular cross-talk networks and regulate in an autocrine and/or a paracrine manner the various stages of normal human hematopoiesis [69]. Thus, this new role of GCs could be particularly relevant if specific chemokines / cytokines were found to be differentially expressed and affecting proliferation or differentiation of erythroid progenitors in DBA.

Immunomodulatory GCs-inspired drugs tested in DBA

The broad anti-inflammatory profile of GCs probably accounts for their marked clinical effectiveness in many types of disease [70]. Since the pathophysiology of DBA was first attributed to immune dysregulation and putative suppressor T cells [6], different immunomodulatory agents were tested, and showed different efficacy profiles: 6-mercaptopurine, cyclophosphamide, vincristine, intravenous immunoglobulin, and anti-thymocyte globulin were tried in patients and found to be largely ineffective [4]. On the other hand, in 10 out of 20 cases treated with a combination of cyclosporine and corticosteroids, transient responses were observed, whereas cyclosporine alone has been reported to cause a sustained response in 2 out of 10 patients [71-78]. Interestingly, the mechanisms of action of the two groups of drugs are different: the drugs of the first group (6-mercaptopurine, cyclophosphamide, vincristine, intravenous immunoglobulin, and anti-thymocyte globulin) have been shown to directly target the T cells, inhibiting their proliferation [79-83], whereas cyclosporine is known to have a milder effect, and modulate the expression of cytokines in T-Lymphocytes and in other cell types [84,85].

Conclusion

Today, 40% of patients with DBA receive steroid treatment, and the prolonged duration of steroid therapy is associated with the risk of side effects [4]; thus, the identification of new compounds that can be used to treat DBA is a priority.

Taken together, the evidence seems to indicate that the therapeutic effect of GCs in DBA could also be linked to the inhibition of cytokine function. In this hypothesis, some cytokines are specifically produced and interfere with normal erythropoiesis in DBA patient erythroid progenitors. This cytokine-mediated hypothesis of DBA is supported by in vitro experiments showing that normal erythroid progenitors secrete cytokines/chemokines, thus regulating hematopoiesis in an autocrine/paracrine manner [69].

SOCS proteins are physiological suppressors of cytokine signaling [86]. Proliferation induction and differentiation arrest caused by the GR mainly depends on mechanisms involving transactivation [18], and by inducing SOCS1 up-regulation in erythroid progenitors [50,55,59], GCs could also act in favor of an inhibition of cytokine pathways. At the same time, this proposition does not exclude the possibility that transrepression (dependent on activations between GR and AP1 or NF-κB) also occurs when erythroid progenitors are subjected to GCs treatment. Since several members of NF-κB family are also expressed [45] in erythroid progenitors, GCs could then inhibit NF-κB-induced cytokines expression, as they do in many other types of cells [41]. Finally, the acceleration of chemokine mRNAs decay by direct interaction between the GR and chemokine mRNAs in human airway epithelial cell line BEAS-2B helps to better understand the rapid action of GCs [68]. This specific degradation of chemokine mRNAs by GCs might partially explain the beneficial effect of GCs treatment in DBA.

These general conclusions on the functional links between cytokines and glucocorticoids should thus be confirmed in the specific field of DBA, in order to determine if drugs targeting cytokines could indeed pave the way for new treatments in DBA.

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