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Diamond Blackfan Anemia Treatment: Past, Present, and Future

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Abstract

Despite significant improvements in our understanding of the pathophysiology of Diamond Blackfan anemia (DBA), there have been few advances in therapy. The cornerstones of treatment remain corticosteroids, chronic red cell transfusions, and hematopoietic stem cell transplantation, each of which is fraught with complications. In this article, we will review the history of therapies that have been offered to patients with DBA, summarize the current standard of care including management of side effects, and discuss novel therapeutics that are being developed in the context of the research into the roles of ribosomal haploinsufficiency and p53 activation in Diamond Blackfan anemia.

Introduction

> Sometimes it takes faith to keep on supporting enthusiastically a patient with incurable disease. In this instance, some patients undergo spontaneous remissions, others benefited by newly available drugs.[1]

Diamond Blackfan anemia (DBA) was originally described in 1936 by Josephs in a review of anemia in infancy and childhood[2] and was further categorized as a congenital hypoplastic anemia by Diamond and Blackfan in 1938.[3] Years later, Diamond, Allen and Magill published a 25-year study of what they termed "congenital (erythroid) hypoplastic anemia."[1] This article, the source of the quote above, provides a history of therapies for DBA. In addition, the authors reviewed the history, physical examination, laboratory data, and natural course of thirty patients who carried the diagnosis defined by (1) normochromic, normocytic anemia developing in early childhood (they did not recognize the frequency of macrocytosis); (2) deficiency of red cell precursors in the bone marrow; and (3) absence of clinically significant leukopenia or thrombocytopenia (they did not note the relatively frequent thrombocytosis). The authors also discussed various therapies that were attempted in these patients. As they write, "even though there was no detectable deficiency, all of the usual hematinics (including ferrous sulfate, crude liver extract, folic acid, vitamin B12,

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vitamin B complex, citrovorum factor, cobalt, testosterone, bone marrow powder, pentonucleotide, ventriculin, and copper) were tried in this group of patients in an effort to stimulate erythropoiesis." These therapies were uniformly unsuccessful.[1]

Success with corticosteroids was first noted in 1951 by Gasser,[4] and a number of the thirty patients in the study of Diamond et. al responded to corticosteroid therapy.[5] Of the thirty patients, twenty-two had one or more therapeutic trials with the hormone. Of these, twelve enjoyed a sustained corticosteroid-induced remission; there were no differences noted in age of disease onset, gender, family occurrence, or degree of hypoplasia between the responders and the non-responders. However, they did note that patients who responded had a much shorter duration of anemia prior to their trial of cortisone. Eighteen of the original thirty patients failed to respond at any time to any form of therapy and continued to receive regular transfusions. Eight of the eighteen patients underwent splenectomy but no improvement in the rate of remission was seen. Another six of the eighteen patients had spontaneous remissions. As Diamond said in the comments at the end of the study, "those patients unresponsive to corticosteroid therapy must not be considered hopeless." The frequency of spontaneous remissions remains a mystery and an important clue to the pathophysiology of the illness.

More than half a century after the original report of their efficacy, corticosteroids remain the mainstay of therapy for patients with DBA. In this paper, we will outline the current treatment options for patients, review the therapies that have been attempted in the past and ultimately abandoned, and discuss some of the newer therapeutic approaches now being explored in DBA.

Current State of Therapies

In 2008, a group of veteran clinicians established a consensus for the diagnosis and treatment of DBA.[6] This document remains the gold standard for therapeutic decisions. Patients with DBA usually present with severe anemia and are stabilized with red blood cell transfusion. Corticosteroids are then considered the first line of medical treatment, after transfusion. Approximately 80% of patients will initially respond to steroids although there is currently no way to select the patients who will so respond.[6] Increases in hemoglobin are usually seen within two to four weeks of administering corticosteroids at a dose of 2 mg/kg/day. The dose is then titrated to minimize side effects while maintaining transfusion independence. The dose can vary considerably between patients. Some are eventually able to stop completely without redevelopment of anemia (state of "remission").

Remission in patients with DBA is not an uncommon event. The male to female ratio of patients entering remission is equal. The actuarial likelihood of entering remission is approximately 20% by 25 years of age. Steroid responsiveness is not a prerequisite for remission as even patients receiving transfusion therapy have entered a remission state.[6] There is no obvious phenotypic or genotypic difference between remission and non-remission patients.[7] Thus, the mechanism of action, the variable response, and the causes of partial or complete remissions remain unknown.

The mode of action of corticosteroids in DBA is particularly obscure. Apoptosis at the progenitor level appears to be the cause of the anemia,[8] and corticosteroids appear to have a non-specific anti-apoptotic effect in erythroid progenitors particularly at the colony-forming unit-erythroid (CFU-E)/proerythroblast interface.[9] In *in vitro* cultures of hematopoietic progenitors, corticosteroids increase the accumulation of progenitor cells[10] and cells capable of erythroid colony formation.[11] Dexamethasone has been shown to induce the expression of genes found in immature erythroid cells while decreasing the expression of genes specific to non-erythroid hematopoietic differentiation.[11] Taken

together, these data suggest that, like androgens, corticosteroids broadly promote erythropoiesis at the progenitor level, and the clinical response of patients with DBA would appear to confirm this. Interestingly, dexamethasone has been shown not to affect the expression of *RPS19*[11] which suggests that the effects of corticosteroids on erythropoiesis are independent of ribosomal dysfunction.

Patients with DBA have long been recognized to have a profound decrease in the number of proerythroblasts in the bone marrow. A novel mouse model using *rps19*-targeting shRNAs appears to localize the erythroid defect at the CFU-E/proerythroblast transition.[9] This is of particular note because it is consistent with earlier work suggesting that the anemia might be due in part to dysfunctional CFU-E. Corticosteroids partially improve the sensitivity of these cells to erythropoietin[12] which might explain, in part, their mechanism of action. Ongoing work is focused on developing drugs that are more specific and potent than steroids at increasing production of CFU-E in patients with Diamond Blackfan anemia. Flygare et al have identified a small number of physiologically relevant glucocorticoid receptor target genes and are currently screening for drugs that are predicted to stimulate the same transcripts as corticosteroids.[13]

The deleterious side effects of corticosteroids are broad and damaging. While a comprehensive review of these is beyond the scope of this paper, some should be mentioned. Adverse complications of long-term steroid therapy were noted in 19.7% of patients from the French registry of DBA.[14] These included fractures, hypertension, diabetes mellitus, growth retardation and one infection related death. The DBA Registry of North America reported 12% of patients with cataracts and 22% of patients with pathologic fractures.[15] The current recommendations are that the start of steroids should be delayed to one year of age if possible to minimize the impairment of growth and neuromotor development as well as the blunting of the acquisition of immunity to live vaccines.[6] Supportive care and prophylaxis as well as careful monitoring for side effects including regular ophthalmologic exams, bone densitometry determinations, and accurate growth charts are essential for patient care.

Patients who do not respond to steroids or are unable to tolerate them may require chronic transfusion therapy with the goal of maintaining hemoglobin levels above 8 g/dl to allow for adequate growth and development.[6] The disadvantages of chronic transfusion therapy include the need for intravenous access, repeated exposure to blood products, and iron overload. As the body’s capacity to sequester excess iron is overcome, iron accumulates and damages vital organs including the heart, liver, pancreas, thyroid and other endocrine glands. The effects of iron overload, particularly on the heart and liver, can be fatal.

Without chelation, the hepatic iron concentration in infants with DBA receiving chronic monthly transfusion therapy would be predicted to rise from an acceptable range (3–7 mg/g, dry weight) to the high risk range (> 15 mg/g dry weight), in one year.[6] Direct methods of assessment of iron burden have included liver biopsy, superconducting quantum interference device (SQUID), and magnetic resonance imaging (MRI); MRI has supplanted the other modalities. Patients with DBA who receive chronic transfusions are routinely screened for iron overload because the rate of iron accumulation and the degree of toxicity varies from patient to patient. There are three iron chelating agents in use worldwide. Two are approved in the United States: deferoxamine (Desferal®), which is administered parenterally and deferasirox (Exjade®), which is administered orally. A second oral agent, deferiprone (Ferriprox®) is approved in Europe,[16] but is a weak iron chelator. A report of fatal agranulocytosis after deferiprone therapy in a child with DBA and other similar but unreported episodes have led to the recommendation that it be avoided in patients with DBA

except in extreme cases of cardiac iron overload and congestive cardiomyopathy[17] and then in tandem with deferoxamine. Clearly deferasirox is the preferred iron chelator.

Transfusion dependence is considered an acceptable indication for a matched sibling donor hematopoietic stem cell transplantation (HSCT). HSCT is the only definitive treatment for the hematologic manifestations of DBA and its role in management is evolving as outcomes for both matched sibling and alternative donor transplants improve.[18] In the 2008 consensus document, it was recommended that HLA-matched related donor transplant be considered for patients prior to the age of 10 years, particularly for patients who were transfusion dependent. Unrelated HSCT is recommended only in the instance of bi- or tri-lineage cytopenia and/or progression to myelodysplasia or AML.[6] With the significant improvements in the outcome of alternative donor HSCT since 2000,[18] these recommendations will likely change at the next consensus conference.

As of December 1st 2009, fifty five patients in the DBA registry had undergone an allogeneic sibling or alternative donor HSCT. For sibling HSCT performed prior to 9 years of age, survival is 90.0±9.5% whereas those older than 9 years of age had a survival of 70.0±11.6%. Survival for alternative donor HSCT was 23.1±11.7% prior to 2000 and 85.7±13.2% since 2000. Alternative donor recipients who were HLA matched molecularly had better overall survival rates than those who were matched serologically.[19] Five patients with DBA have been transplanted at Children's Hospital Boston since 2001. Four of these patients received matched sibling HCST and one patient received an unrelated HSCT. One of the patients, who received a sibling transplant, died from veno-occlusive disease and multi-organ failure while the remainder are all clinically well. [L. Lehmann, personal communication] These data suggest that the role of transplant in DBA will need to be revisited with a particular emphasis on the appropriate age to transplant patients, the role of iron burden, and other improvements that affect the success of HSCT.

In summary, corticosteroids, chronic transfusions, and hematopoietic stem cell transplantation are the currently utilized therapies for patients with Diamond Blackfan anemia. Recommendations for each of these modalities were summarized in a 2008 clinical consensus document. With existing treatments, the overall survival of patients, as reported by the DBA Registry, is 75.1% at 40 years of age.[15] Clearly, there is a need for additional or alternative therapies given the survival rates and the toxicities associated with each of the existing therapies. The abundance of scientific advances in the field, which are summarized elsewhere in this compilation, is expanding the list of potential treatments. Before exploring these possibilities, it is worth revisiting the therapies that have been tried in the past so that the lessons learned can be applied to future drug development.

Summary of Past Therapies

From its early description by Diamond, Allen and Magill as a "rare entity in which subnormal erythropoiesis produces a gradual but profound anemia,"[1] DBA has been recognized as a disorder of abnormal erythropoiesis. *In vitro* studies of erythropoiesis have shown a moderate to severe deficiency of erythroid burst-forming units (BFU-E) and colony-forming units (CFU-E).[12,20,21] Therefore, many of the earliest therapies were directed towards stimulating erythropoiesis either directly or via modulation of hematopoietic growth factors.

Erythropoietin, the key growth factor in erythropoiesis, was administered to ten patients with only one having a transient response.[22,23] It is now known that patients with DBA have elevated serum and urine erythropoietin levels, and exogenous erythropoietin does not have a role in the treatment of the disease. Therapies with broad-acting, recombinant growth

factors including interleukin-3 and stem cell factor have also failed to show consistent benefit.[6]

Androgens have long been recognized as having effects on erythropoiesis and their mechanism of action includes increasing erythropoietin production and stimulating erythroid progenitor cells.[24] Hemoglobin levels increase in normal males and in patients treated with androgens for non-hematologic conditions which suggests that androgens have a much more profound effect on erythropoiesis when compared to corticosteroids in normal individuals.[24] Given these effects and their use in aplastic anemia, androgens have been administered to several DBA patients. The results have been variable. Of note, there have been two DBA patients reported with malignant tumors who had received previous treatment with the combination of corticosteroids and androgens.[25] Given these reports, the lack of consistent response, and the significant toxicities associated with androgen therapy, this modality is no longer considered appropriate in DBA.

In addition to disordered erythropoiesis, the pathophysiology of DBA was attributed by some investigators to immune dysregulation and putative suppressor T cells.[26] This was subsequently shown to be likely due to an artifact of transfusion.[27] Because of this initial hypothesis, cyclosporine was administered to several patients with steroid-resistant DBA. The combination of steroids and cyclosporine did lead to transient responses and cyclosporine alone has been reported to cause a sustained response in a handful of patients but there is no clear way to predict these unique responders.[28–35] The response to cyclosporine in a congenital disorder of the stem cell with no evidence of immune dysfunction should encourage clinical investigators to eschew assignments of pathophysiological mechanisms on the basis of administration of drugs with very broad mechanisms of action. Other immunomodulatory agents including 6-MP, cyclophosphamide, vincristine, IVIg, and ATG have been tried in patients and found to be largely ineffective.[6]

The pathophysiology of DBA is now increasingly attributed to ribosomal dysfunction, and mutations in ribosomal genes have been identified in approximately 50% of patients.[36] The 5q- syndrome is a distinct subtype of myelodysplastic syndrome (MDS) characterized by a severe macrocytic anemia.[37] *RPS14* was identified as a 5q- syndrome gene in an RNA interference screen of each gene within the 5q- syndrome common deleted region (CDR).[38,39] Although patients with the 5q- syndrome have historically been maintained on chronic transfusions, it has now been shown that a large percentage of these patients respond quite dramatically to the thalidomide derivative, lenalidomide. In a Phase 2 trial in low risk MDS patients with 5q deletions, lenalidomide treatment decreased transfusion requirement in 76% of patients, and 61% of patients had a complete cytogenetic response.[40] Although lenalidomide is known to have effects via immunomodulation as well as anti-angiogenic and direct anti-tumor activity, its exact mechanism of action in MDS remains unknown.[41] Because of the similarities between DBA and the 5q- syndrome, there has been significant interest in whether lenalidomide would be an effective therapy in patients with DBA and a pilot study is open in the US. This study is being conducted cautiously as clonal eradication in DBA could potentially lead to aplastic anemia. The experience of lenalidomide therapy in the 5q- syndrome should also influence clinical investigators to be wary of assigning pathophysiological mechanisms on the basis of clinical trials with broadly active but fundamentally mysterious drugs.

Three additional drugs have been used with some limited success in patients with Diamond Blackfan anemia. Metoclopramide is known to induce the release of prolactin from the pituitary gland. A woman with severe macrocytic anemia was noted to have improvement in her anemia during pregnancy and while breast-feeding (when prolactin levels are elevated).

[42] The drug was administered to 9 patients with DBA; three had clinically significant responses.[42] However, in a follow-up prospective study of 33 patients, only two patients had a partial response to the therapy.[43] The combination of metoclopramide, an inexpensive and generally well tolerated drug, with corticosteroids remains to be tested.

Another unique patient was the source of the observation that valproic acid might have a role in the treatment of DBA.[44] This 19 year-old female with DBA had been previously treated with prednisone, methotrexate, cyclosporine, and metoclopramide but was still requiring transfusions. During an acute illness, she developed generalized tonic-clonic seizures and was started on valproic acid. Subsequently, she has required no further blood transfusions.[44] Valproic acid is a histone deacetylase (HDAC) inhibitor and is also known to induce the expression of fetal hemoglobin[45] Whether the valproic acid influenced the remission or whether the remission was ‘spontaneous’ is, of course, unknown.

Finally, based on the theory that inefficient translation, which would be expected with ribosomal dysfunction, might be the main cause of the severe anemia,[46] a group in the Czech Republic decided to test the effects of leucine in DBA. The one patient reported had a complete response with no side effects from therapy.[47] A larger trial is underway in the Czech Republic and a trial has now been approved in the United States. Leucine, an amino acid which is known to modulate protein synthesis, is believed to act through the mTor pathway. A further discussion of the potential role of the mTor pathway in the pathophysiology of DBA will be reviewed in this compilation.

Future Directions for Therapy

As detailed in other articles in this collection, tremendous progress has been made in elucidating the pathophysiology of Diamond Blackfan anemia although many questions remain. It is clear that ribosomal haploinsufficiency plays a critical role in DBA[48] and dysfunction of ribosomes and ribosomal RNA contributes to several other bone marrow failure syndromes including Shwachman-Diamond syndrome, dyskeratosis congenita, cartilage hair hypoplasia, Treacher Collins syndrome, and the 5q- syndrome.[36] A mutation in a ribosomal protein gene would be expected to have widespread and diverse effects throughout an organism and indeed patients with DBA and the other ribosomopathies have many congenital anomalies. These too may provide insights for future drug development.

How might a ribosome gene mutation lead to the manifestations of DBA? A leading hypothesis is that haploinsufficiency of one ribosomal protein leads to disrupted ribosome biogenesis and an accumulation of free ribosomal proteins that bind MDM2, a repressor of p53. The consequent activation of p53 leads to apoptosis of progenitors, which ultimately leads to anemia.[49,50] Erythroid progenitors are apparently more sensitive to this mechanism than are granulocyte/macrophage or megakaryocyte progenitors.[51] Another potential mechanism is defective maturation of ribosomal subunits that could delay translation of globin genes, resulting in a relative excess of free heme, which would also lead to erythroid-specific apoptosis and anemia.[52] The problem here is that hemoglobin accumulation in the erythrocytes of DBA patients is quite normal. Alternative mechanisms include pathogenic functions for the aberrantly accumulated ribosomal precursors and aberrant translation by defective ribosomes.[53] Murine models are being developed to inform the biology of ribosomopathies and to serve as critical resources for the development and testing of novel therapies.

In a murine model with a heterozygous missense mutation in *rps19*, induction of p53 and p53 target genes was identified in the hyperpigmented foot pads of the mice.[49] When the *rps19* mutant mouse line had one allele of p53 genetically inactivated, there was an increase in RBC count and decrease in MCV. Homozygous inactivation of p53 in *rps19* mutant mice

fully corrected the hematologic phenotype.[49] Another murine model involves the conditional deletion of a set of genes on 5q including *rps14.* These mice develop a severe macrocytic anemia, consistent with the phenotype of Diamond Blackfan anemia and the 5q- syndrome.[54] When these mice were crossed with p53 null mice, there was a complete rescue of the erythroid phenotype.[54]

Finally, a murine model of Treacher Collins syndrome offers even more tantalizing clues to the potential management of patients via modulation of the p53 pathway.[55] Treacher-Collins syndrome is an autosomal dominant condition which includes characteristic craniofacial changes[56] that arise from symmetrically and bilaterally diminished growth of the structures derived from the first and second pharyngeal arch, groove, and pouch.[57] In 1996, *TCOF1* was identified as the gene responsible for TCS; *TCOF1* encodes a protein known as Treacle.[58] Mice haploinsufficient for *tcof1* exhibit diminished production of ribosomes and this deficiency correlates with decreased proliferation of both neural ectoderm and neural crest cells.[59] Interestingly, a study by Jones et al [55] showed that chemical and genetic inhibition of p53 activity in these mice can prevent the craniofacial abnormalities.

Studies in mice with conditional inactivation of *rps6* have further elucidated the mechanism of MDM2-mediated induction of p53 by ribosomal haploinsufficiency.[50] Conditional deletion of *rps6* in murine liver inhibited 40S (but not 60S) ribosomal biogenesis, and the liver in affected mice did not regenerate normally following partial hepatectomy due to cell cycle arrest.[60] The increased free rpl11 that was generated by the mechanism was found to be due to the increased translation of rpl11 mRNAs through derepression of 5'-TOP mRNA translation.[50] If specific agents that target the signaling components involved in rpl11 upregulation and/or that regulate 5'-TOP mRNA translation could be developed, there is the potential to alleviate the block in ribosomal biogenesis without involving other pathways involved in p53 induction.

In summary, our increasing understanding of the pathophysiology of Diamond Blackfan anemia is leading to new therapeutic avenues potentially involving the modulation of p53 and mTOR. Although all of these pathways would be expected to have widespread consequences, there might be a therapeutic window that can be achieved as evidenced by the successful inhibition of p53 in the Treacher-Collins model with pifithrin-α, a chemical inhibitor of p53-dependent transcription and apoptosis.[61] Furthermore, data from the open trials with leucine and lenalidomide as well as trials with HDAC inhibitors in other red cell disorders may also provide useful insights for directing future therapies.

Finally, stem cell transplantation remains the only curative option for patients with DBA which raises the possibility of cure by gene therapy using the patient's hematopoietic stem cells followed by transplantation of the corrected cells. *In vitro* experiments using *RPS19* overexpression in DBA cells showed a three-fold increase in the formation of BFU-E colonies and improvement of the erythroid failure.[62,63] Although quite preliminary, these results suggest that further work on gene therapy using *in vivo* models needs to be pursued.

Conclusions

Since its original description 75 years ago, Diamond Blackfan anemia has challenged physicians and scientists with its variable clinical course and response to treatment. Many therapies have been tried over the years with inconsistent success and the cornerstones of therapy remain chronic red blood cell transfusion, corticosteroids and hematopoietic stem cell transplantation. Diamond Blackfan anemia has also served as a model for a new class of diseases, now known as ribosomopathies, characterized by dysfunction of ribosome

biogenesis. Our hope is that as progress is made elucidating the pathophysiology of this fascinating disease, there will be simultaneous advances in the discovery of new therapeutic targets and drug development.

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