

* CHAPTER 5

Diamond-Blackfan anaemia, a constitutional erythroblastopenia

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1. Introduction

Erythroblastopenia is characterised by the absence or less than 5% of erythroid precursors in an otherwise normal bone marrow (the granulocyte and megakaryocyte lineages are usually normal). Erythroblastopenia results in a normochromic, normocytic or macrocytic, aregenerative anaemia.

Erythroblastopenia may be congenital, with only one disease described, the Diamond-Blackfan anaemia (DBA) or it can be acquired, which is due in most cases to erythrovirus (parvovirus) B19 infection, or may be associated with immunologic disorders. This chapter will focus on the congenital erythroblastopenia - Diamond-Blackfan anaemia.

2. Clinical presentation and laboratory findings

2.1 Clinical presentation

DBA, is a rare disorder with a prevalence of 5-7 cases per million live births (1-3). Males and females are equally affected. No seasonal or environmental factors have been observed. There is no founder effect and DBA can occur in all human groups. In the vast majority of cases (95%), diagnosis is made before the age of 2 years, with a median age at diagnosis of 3 months. Initial clinical manifestations are those of an isolated anaemia during infancy: pallor, shortness of breath while suckling, failure to thrive, systolic murmur. According to the age at the first blood count and to individual variations, anaemia can be moderate or severe. It is usually macrocytic with persistently low reticulocyte counts. Moderate thrombocytopenia and/or neutropenia, or thrombocytosis may be of no clinical or diagnostic relevance. Liver and spleen size are normal.

Bone marrow smears depict the absence or rarity of erythroid precursors ($\leq 5\%$ of nucleated cells), which have a normal morphological appearance. Bone marrow cellularity is normal and other haematopoietic lineages show no maturation defects. Anaemia is present at birth in only 15% of cases and foetal hydrops has rarely been reported, suggesting that erythropoiesis is spared *in utero*, maybe because it is transiently rescued by maternal or placental factors with a post-natal switch from effective to ineffective erythropoiesis.

Congenital anomalies are associated with the anaemia in 10-40% of DBA-affected individuals: intrauterine growth retardation and growth impairment are common features and many patients will have to cope with a small stature, often aggravated by long-term steroid therapy or iron overload. Small stature is considered part of the malformation syndrome, which can affect the cephalic area (microcephaly, cleft plate, Pierre Robin syndrome, hypertelorism, high arched palate, etc.), thumbs and

upper limbs (triphalangeal thumb, thumb duplication or hypoplasia, radial hypoplasia, etc.) but also the heart, kidneys, urogenital tract and bones (2). Mental retardation may be observed. Such anomalies may be isolated or associated with a wide variety of possible combinations. None is DBA-specific and many of them also occur in other constitutional cytopenias, such as Fanconi anaemia and TAR syndrome.

An investigation should also be conducted into any family history of obvious DBA, moderate or transient anaemia, or malformations.

2.2 Laboratory findings

Regenerative anaemia and erythroblastopenia, the main laboratory findings, are usually associated with an increase in erythrocyte adenosine deaminase (eADA) activity which, although nonspecific, is found in 90% of DBA-affected individuals but, for obvious reasons, can only be assessed in non-transfused patients (4-7). eADA is a key enzyme in purine metabolism but its relevance to DBA pathophysiology remains unexplained. Although there is a degree of overlap when comparing a control population with a population of DBA patients, it is commonly accepted that increase in eADA activity above the threshold of 1.70 nmol/min/mg haemoglobin is strongly suggestive of DBA (4). eADA should also be systematically assessed in family members. Persistence of high levels, according to age, of haemoglobin F or i antigen, which are markers of foetal erythropoiesis, is also generally observed in DBA patients but has no diagnostic value. After obtaining informed consent, DNA samples from the patient and family members should be stored for further molecular studies, including ribosomal protein gene mutation screening.

2.3 Differential diagnosis

At the same time, several other diagnoses have to be ruled out. Chronic erythroblastopenia during the first weeks or months of life, which requires specific management, may be caused by transplacental contamination by erythrovirus (parvovirus) B19 when a maternal primary infection has occurred during pregnancy. Serological testing of maternal and newborn blood and bone marrow (IgG and IgM specific antibodies) and viral PCR are mandatory. Transient erythroblastopenia of childhood (TEC), which is presumably of viral origin, can be observed early in infancy but will resolve spontaneously. However, TEC has been reported in healthy DBA siblings, which along with other arguments raises the possibility of a subtle sensitivity towards an impairment of erythropoiesis in some apparently unaffected family members. Many physicians determine the constitutional karyotype and include a chromosome breakage test or a Western blot to look for the monoubiquitination of FANCD2, in order to rule out Fanconi anaemia.

3. Treatment

3.1 Oral steroids

In nearly all cases of DBA one or several blood transfusions will initially be needed to counter severe anaemia in early infancy. Once the diagnosis has been established with a high degree of probability, oral steroids are the first-line therapy, which can restore effective erythropoiesis leading to transfusion independence in more than 60% of patients (2). Recent guidelines suggest that steroids should not be used before one year of age, in order not to harm growth, which is very active at this period of life. High-dose steroid therapy carries a high risk of toxicity and risk of sepsis and should not be used (8). The initial dose for conventional oral steroid therapy is usually 2 mg/kg/day of prednisone or equivalent. A rise in reticulocyte count occurs within 10-15 days if the patient responds. If this rise occurs, the daily dosage should be cautiously tapered in an attempt to reach persistent transfusion independence at a dose ≥ 0.5 mg/kg/day, which can be prescribed on alternate days. Patients who require doses above this threshold should not be maintained on long-term steroid therapy, in view of its deleterious side effects.

The simultaneous use of steroids and transfusions is in our experience disappointing and carries the risks of both therapies. Some paediatricians advocate the transient discontinuation of steroids during periods of life that are critical for growth, such as the first year and the prepubertal period: a transient switch towards regular transfusions can result in a growth spurt and lead to a more tolerable final height. Steroid sensitivity or resistance may vary with time in a given individual, as may the threshold of steroid sensitivity, which in some adults can decrease to very low, although necessary, doses such as 5 mg/day. DBA patients can switch from steroid responsiveness to resistance, or inversely (2). Such unpredictability leads many physicians to test the steroid effect again in previously resistant patients, especially before taking crucial decisions, such as bone marrow transplantation. Some patients can be completely weaned off all treatment. Such treatment independence, which often occurs in teenagers, can be lifelong or interrupted by new periods of treatment when needed. Treatment independence can in no case be considered as complete remission or cure: in most patients erythropoiesis continues to show anomalies such as persistent moderate anaemia, macrocytosis, and high eADA levels. The mechanisms that allow steroids to restore effective erythropoiesis are still a matter of debate. *In vitro* dexamethasone, when added to the culture medium, can enhance the Epo sensitivity of erythroid progenitors from both controls and DBA patients, resulting in increases in cloning efficiency, colony size and haemoglobinisation (9). Glucocorticoid, Epo, and stem cell factor (c-kit) receptors

have been shown to act in synergy and to increase erythroid cell proliferation (10). Similarly, dexamethasone increases the transcription efficiency of several genes involved in erythropoiesis, such as *c-kit*, *AML-1*, *SCL*, *MML*, but is devoid of any effect on *RPS19*, *NFE2* and *EKLF* (11).

The lack of correlation between *in vitro* data and the response to steroids *in vivo* has been a constant hindrance to those who attempt to move research from the bench to the bedside (9, 12, 13).

3.2 Transfusion and iron chelation

Patients who are steroid-resistant enter a regular transfusion program. Due to the risk of bloodborne infections and of iron overload, some physicians allow haemoglobin values to decrease to a critical threshold before deciding on the next transfusion. We aim to optimise the patient's quality of life. Most patients have an individual haemoglobin threshold below which they feel tired, depressed or uncomfortable, and this threshold needs to be defined. The amount of packed red blood cells transfused and the intervals between transfusions must be individually tailored to allow the patient to pursue his or her school or working life, social and family activities, avoiding a succession of up and down periods. Long-term transfusion programs necessitate iron chelation, for which deferoxamine is the reference therapy. This therapy is burdensome (overnight subcutaneous infusions 5-7 times/week) and must be instituted early in life, as soon as successive serum ferritin measurements are ≥ 1000 ng/mL in the absence of overt inflammation. Subsequently, liver iron content and blood ferritin have to be regularly monitored and compliance must be constantly encouraged in patients, specifically during adolescence and early adulthood. Due to unpredictable side effects, mostly neutropenia, prescription of the oral chelator deferiprone is mostly limited to patients who have experienced side effects of deferoxamine or who fail to comply with regular subcutaneous injections. However, combination of the two treatments has in some individuals resulted in better acceptance by decreasing the number of infusions or has allowed partial correction of severe iron overload in patients originating from developing countries where the high cost and uncertain availability of drugs have impeded regular chelation. The association of deferoxamine and deferiprone is also the most preferred treatment of cardiac haemochromatosis, which can be easily diagnosed by T2* MRI (14). Recently, a new potent oral iron chelator, deferasirox, has been shown to be effective in patients on a regular transfusion program, including several DBA patients (15). Its use is a major step in the management of DBA, increasing quality of life and protecting against irreversible organ damage in patients who are reluctant to accept subcutaneous deferoxamine.

3.3 Haematopoietic stem cell transplant (HSCT)

HSCT is currently the sole treatment that can cure DBA, restoring a persistent normal erythropoiesis of the donor type. The consensus is that an HLA-identical sibling should be used as a donor in a patient who requires regular transfusions. HSCT can restore erythropoiesis in nearly 90% of cases. Familial cord blood can also be used. Nevertheless, the choice of donor among HLA-identical siblings should be made carefully in order to eliminate individuals affected with a mild form of the disease or silent phenotype (macrocytosis and/or elevated eADA and/or mutation in *RPS19* gene without anaemia). Matched unrelated donors should be considered only in a patient with severe haematological complication (bone marrow aplasia, myelodysplasia or leukaemia). Selection of patients for HSCT and the age when it should be performed are still problematic, because of the persistence of risks, albeit decreased, related to the procedure and the unpredictability of the clinical course in each individual, and the possible emergence of new treatments over the next decade. Some physicians who perform HSCT early in the patient's life have achieved remarkable results (Charlotte Niemeyer and Jörg Meerpohl, Maria Daniella Arturi Foundation, 4th International Consensus Conference, New York, 2003). Others are more reluctant and limit the indications to treatment intolerance and/or haematological complications such as bone marrow hypoplasia, myelodysplasia and leukaemia, despite the increased risk of graft failure or complications with a long-standing transfusion program and with increasing age.

3.4 Other treatment approaches

Other treatments have been shown to be effective in only a few patients or in individual case reports. The growth factor interleukin-3 (IL-3) has resulted in treatment independence in 5-10% of patients in small study series. Interestingly, two European patients developed effective and treatment-free erythropoiesis several years after cessation of therapy (16). Further therapeutic trials with IL-3 have been cancelled due to the cessation of the industrial production of the drug, which was designed for other indications.

Cyclosporine, alone or in combination with steroids, has proved effective in some cases (17, 18). Metoclopramide, a commonly used anti-emetic drug whose effect is mediated by an increase in endogenous prolactin synthesis, was recently shown to be effective in 3 out of 9 evaluable patients in a preliminary trial (19) and in one case report (20). However, a therapeutic trial in a more consistent cohort of transfusion-dependent patients gave more disappointing results, with less than 10% of partial responses (21). Nonetheless, this low-cost and well-tolerated treatment should be evaluated in all transfusion-dependent patients. In some individuals a synergy between metoclopramide and low doses of steroids has been clearly

demonstrated. Individual reports of responses with valproic acid (22) or rituximab (23) are for the moment anecdotal. Gene therapy is still part of preliminary research programs but is potentially of promise for patients with identified mutations (24-26).

4. Complications

The clinical course of DBA through the life of a given patient is unpredictable. Severe complications are in most cases treatment-related, due to the side effects of long-term steroid use, iron overload, bloodborne infections and adverse effects of HSCT. However, over the last decade there has been an increase in attention paid to the deleterious effects of long-term steroid therapy, and in awareness of the prevention of iron overload. Also, significant progress has been made in the safety of transfusion and management of HSCT. Young DBA-affected individuals even if persistently treatment-dependent can enjoy an improved quality of life with a normal social and familial integration, despite the burden of a chronic disease. Disease-related complications are less frequent, but will unfortunately become more prominent in the decades ahead as survival improves through individualised management. Pancytopenia due to bone marrow aplasia or hypoplasia, or in some cases associated with abnormal morphology of bone marrow cells mimicking myelodysplasia, in the absence of clonal markers, have been reported in some patients and in some cases resolve spontaneously or under steroid therapy. Nevertheless, haematological malignant diseases, as well as solid tumours, may occur, with a risk of around 1-4% (2, 3, 27). Haematological malignancies mostly include myelodysplasia and AML but also ALL, lymphomas and Hodgkin's disease (2, 3, 27, 28). Solid tumours vary widely and include osteosarcoma (29), gastric or other digestive carcinomas, hepatic carcinoma, and breast cancer (2, 3, 27, 28). It has been suggested that in many cases such cancers occur earlier in life than in a control population and that their treatment is difficult because of the haematological chemotherapy-related toxicity associated with DBA. Cautious follow-up is mandatory in all DBA patients in order to detect such events early, but it has to be emphasised that the risk is low, when compared to other models of constitutional cytopenias such as Fanconi disease and Shwachman-Diamond syndrome. Lastly, it has been suggested that the risk of osteosarcoma is enhanced by growth hormone (GH) treatment for growth impairment (29). GH has been shown to be effective in initiating growth spurts in DBA-affected individuals but its use should be considered carefully.

Medical advances have reduced infertility among DBA-affected women and their increased self-esteem and wish to have children have led to more pregnancies. With this increase has emerged evidence of the potential hazards of pregnancy in DBA-affected women. Spontaneous abortion, pre-eclampsia, foetal death, and premature

delivery occur in nearly 50% of pregnancies, whatever the foetal status regarding the *RPS19* mutation or anaemia (30). Pregnancy in DBA-affected women should not be discouraged but calls for careful management and follow-up with close cooperation between obstetricians, haematologists and neonatologists.

5. Genetics

Seventy-five percent of DBA cases appear to be sporadic. Familial forms display a dominant mode of inheritance; recessive forms appear to be questionable.

A 1997 report describes the case of a DBA-affected female with malformations, in whom a constitutional balanced translocation 46,XX, t(X;19)(p21;q13) was found (31). As males and females are equally affected, such an observation sheds light on chromosome 19. Study of the DNA of multiplex families and the observation of chromosome 19 deletions in sporadic forms led to the definition of a first DBA locus of 1 Mb (32), and subsequently to identification of a gene. Surprisingly, this gene codes for a ribosomal protein *RPS19* (33). *RPS19* is a highly conserved gene of 11 Kb with 6 exons including exon 1, which is non-coding. Two promoter regions have been identified and shown to act in synergy (34). *RPS19* mRNA has 435 nucleotides (ORF) and codes for a 145-amino-acid protein. *RPS19* has been shown to have prominent nucleolar localisation, suggesting a role of *RPS19* in ribosome biogenesis. Some mutations in DBA patients have been experimentally shown to result in cellular mislocalisation and decreased expression level of the protein (35, 36) in perfect correlation with the three-dimensional structure of *RPS19* (37). The mutations associated with a slight decrease in *RPS19* expression levels and normal nucleolar localisation are all accessible residues clustered within or around alpha helix 3 located in the central position in the structure and thereby impairing the function of the protein without altering its overall fold (class II mutations). In contrast, mutations, which exhibit a dramatic decrease in *RPS19* expression levels alter structural residues affecting the folding of the protein and hence its stability (class I mutations).

However, broad heterogeneity in the genetics of DBA has been shown: *RPS19* gene mutations are only observed in 25% of cases, with a prevalence that is constant in all reported series (33, 38-40). There is great diversity in the nature and localisation of mutations, with a hot spot between codons 52 and 62 (class II mutations (37)). Regarding mutations identified in the *RPS19* gene, no genotype/phenotype correlation has been found as far as familial forms, associated malformations and therapeutic response are concerned. It has, however, been suggested that deletions result in more severe forms (39). All patients are heterozygous and mutations in both alleles are likely to be lethal.

In vitro experiments using fibroblasts or lymphoblastoid cell lines derived from DBA patients have shown that *RPS19* mutations leading to a premature stop codon or abolishing the stop codon result in abnormal transcripts which are eliminated via the NMD (nonsense-mediated decay) pathway, and by nonstop decay, a system that gets rid of mRNAs whenever they risk impairing protein translation (41). The potential involvement of *RPS19* in this system favours the hypothesis of haploinsufficiency in DBA-related cases (42): either only the wild allele is expressed, or the respective expression of both the mutated and the wild alleles is modulated by the NMD system or proteasomal degradation (36). Such a mechanism could be taken into account in order to explain individual variation with time in the degree of anaemia or in the treatment response, and the variety of phenotypic expressions in different family members, for an identical mutation. The same mutation can lead to overt DBA as well as to mild macrocytic anaemia or an isolated increase in eADA. In some individuals, no clinical anomalies or abnormal laboratory values can be observed. Such observations have led to the concept of "silent phenotype" (4). Patients with a silent phenotype and a mutation should be excluded as HSCT donors, as should patients with isolated high eADA values or mild macrocytic anaemia when DBA in the index case is not linked to *RPS19*. Such HSCT can lead to chimerism for non-erythroid lineages, while not rescuing erythropoiesis (43). The wide variation of phenotypic expression complicates genetic counselling, even when a mutation has been identified in an affected adult who wishes to have children or in a child whose parents hope for another pregnancy. However, preimplantation assessment for the mutation, together with HLA testing to ensure compatible cord-blood for transplantation from a disease-free sibling, has already been performed in families where a DBA-affected child presented with an *RPS19* gene mutation (44).

It has recently been suggested that TEC is a mild form of DBA, as the 19q13.2 locus appears to be linked to susceptibility to TEC in some families, although devoid of the *RPS19* mutation (45). This remains hypothetical.

The genetic heterogeneity of DBA has been further emphasised by linkage analysis evidence for another locus, *DBA2*, located on chromosome 8 (8p23.2-23.1) in families with normal *RPS19*, but the gene is still to be defined. However, the same group described recently additional ribosomal protein genes involved in DBA, namely *RPL5*, *RPL11*, *RPS7*, *RPL36* (46), *RPS17* (46, 47), *RPS24* (48). In studying DBA patients with a constitutional 3q deletion, Farrar et al. (49, 50), identified *RPL35a* as a new gene mutated in DBA. Strikingly, not only ribosomal protein genes from the small sub-unit but also from the large sub-unit are involved in DBA pathophysiology. At this time, we can estimate that half of the DBA patients carry a mutation in a ribosomal protein gene. The other half may also carry mutation in

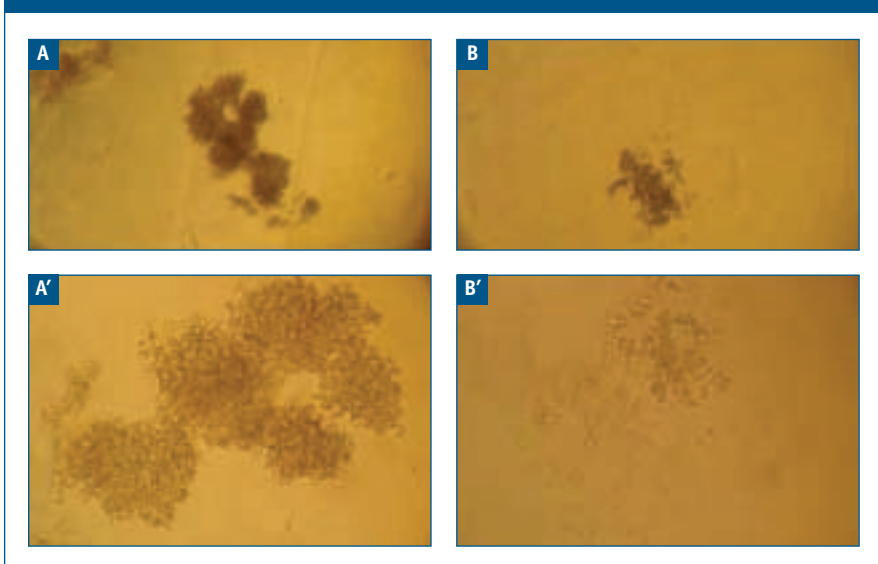
a ribosomal protein gene yet to be identified as a DBA gene, or in a cofactor gene (almost 200 cofactors are involved in ribosome assembly) or in a gene with extra-ribosomal function.

6. Pathophysiology

Seventy years after its initial description (51, 52), DBA pathophysiology is beginning to be explained. First, immune mechanisms have been ruled out (53, 54), as has a microenvironment defect, since control CD34+ cells differentiate normally into the erythroid lineage when cultured over stoma cell layers from DBA patients (55). It is commonly accepted that DBA stems from an intrinsic defect of erythroid progenitors that are unable to achieve normal differentiation (12, 56). However, this intrinsic defect is incompletely elucidated.

Although of no practical relevance for diagnostic purposes, cell cultures have led to major advances in understanding the defect at a cellular level. Erythroid colonies grown in semi-solid medium from blood or bone marrow-derived erythroid progenitor cells are small compared to controls (Figure 1) (9), but have normal or subnormal cloning efficiency, which may, however, decrease with age (57). Blockade of

Figure 1: DBA erythroid precursor colonies in methylcellulose



In vitro comparison of the size of erythroid colonies from peripheral CD34+ cells of a control (A, A') and a patient with DBA (B, B'), at D7 of culture in methylcellulose at an Epo concentration of 2 IU/mL. Magnification X10 (A, B) and X60 (A', B'). Photos: Aurore Crétien

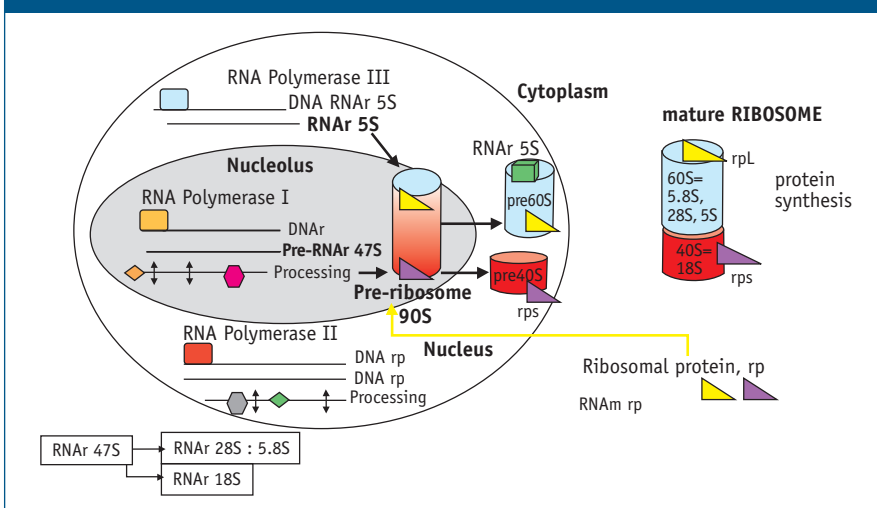
erythroid differentiation and proliferation has been shown to affect immature progenitor cells or BFU-e. Downstream, CFU-e are also subsequently affected (12, 13). A study using a two-phase culture system has localised the defect at the junction between erythropoietin (Epo)-independent and Epo-dependent cells (9). Circulating Epo levels are increased in DBA patients and high-dose Epo treatment is ineffective (58, 59). Taken together, these findings seem to incriminate the Epo receptor (Epo-R) or its signalling pathway. Similarly, an increased propensity of erythroid progenitors to apoptosis during *in vitro* Epo deprivation (60) and in RPS19 deficiency (61-63) has been reported. A defect in the apoptosis pathway may be responsible for the specific erythroid lineage impairment; meanwhile the increased apoptosis may be the result of another defect upstream. However, other experimental studies have shown, using long-term culture-initiating cells (LTC-IC), that differentiation and proliferation can also be impaired in other cell lineages: an early maturation defect with an impact on granulocytic and megakaryocytic as well as erythroid lineages has been identified (55, 57, 64-66). It is noteworthy that the cloning efficiency of CFU-GM cells decreases with aging (57, 65). Such experimental data are consistent with the progression of some patients towards pancytopenia and bone marrow hypoplasia.

Recently, the major advances in the mechanistic understanding of DBA pathophysiology comes from ribosome biogenesis analysis first in yeast (67), and in HeLa cells, then in primary cells from DBA patients (skin fibroblasts, EBV-infected lymphoblastoid cells) (68) (Figures 2 and 3). DBA is now clearly defined as a defect in the ribosomal RNA (RNAr) maturation, which is responsible for an accumulation of immature RNAr. In RPS19 deficiency, the rRNA maturation defect lays at the cleavage site A2 of the internal transcribed spacer 1 (ITS1) in the 3'UTR of 18S rRNA (Figure 3). It leads to an abnormal pre-particle 40S, which is unable to be exported into the cytoplasm due to the binding impairment of some cofactors (67). This process results in a defect in ribosome assembly. Strikingly, the rRNA maturation defect depends on the genetic anomalies identified in DBA. RPS24 deficiency leads to an impairment in the ETS (external transcribed spacer) of 18S rRNA (69).

7. RPS19 and erythropoiesis

Like other ribosomal proteins, RPS19 is involved in protein synthesis but its specific role in normal and impaired erythropoiesis is still to be completely defined. The use of siRNA specific for RPS19 mRNA has been shown to decrease *in vitro* erythroid differentiation and proliferation in erythroid cells derived from cord blood and bone marrow CD34+ cells from normal controls (11, 70). The phenotype is similar to that obtained when using DBA-derived CD34+ cells for erythroid

Figure 2: Eukaryote ribosome synthesis

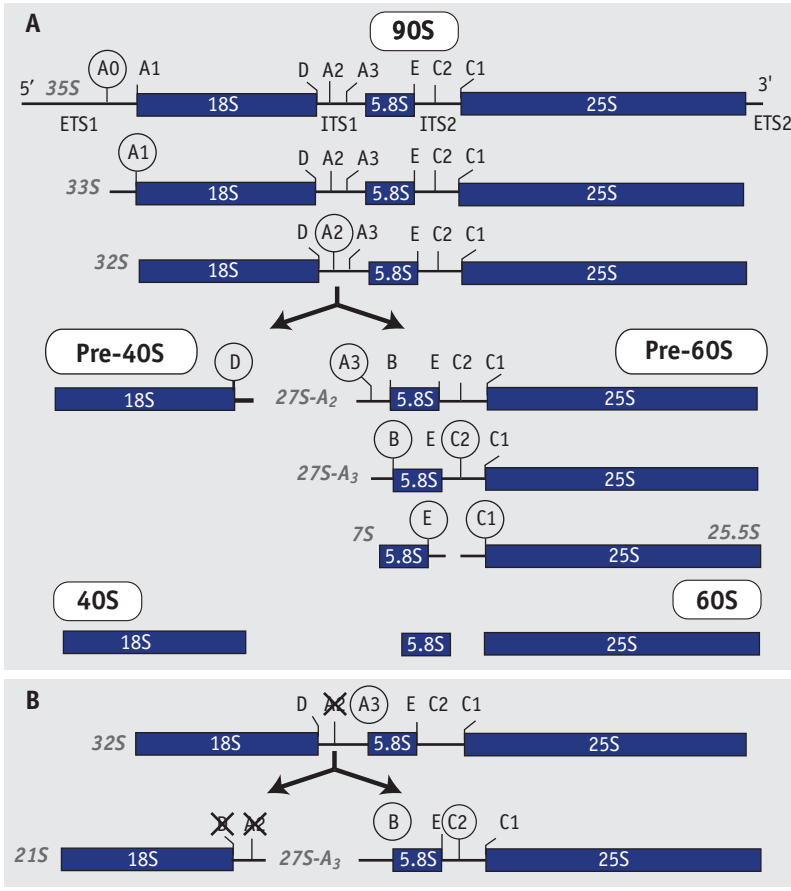


Ribosome assembly. Specific mRNA of each of the 80 rp proteins and 5S rRNA are transcribed in the nucleus from RNA polymerase II and III respectively; the transcription of a DNAr by RNA polymerase I in the nucleolus leads to a big rRNA precursor, the pre-RNAr 47S. The assembly of 5s rRNA, pre-RNAr 47S, and the rp proteins build a bigger ribonucleoprotein complex, the pre-ribosome 90S. After rRNA processing, the pre-ribosome 90S is cleaved in two pre-particles, the 40S and the 60S pre-particles, which are the precursors of the small and the large ribosome subunits. Both pre-particles are exported in different nuclear pores. In the cytoplasm, both small and large ribosome subunits get assembled on the mRNA to be translated and the protein synthesis begins.

cultures and can be reversed by lentiviral transfection of wild-type mRNA (70). The involvement of RPS19 in erythropoiesis has now been demonstrated. The precise molecular mechanism has yet to be fully deciphered.

RPS19 is a part of the small ribosomal subunit (40S) located in the beak, which is a functional domain. Cross-linking experiments have shown that RPS19 binds e-IF2 (71, 72), a factor involved in the initiation of translation, making it likely that RPS19 is implicated in the early stages of protein synthesis. Reported non-ribosomal functions of RPS19 are limited to the chemotactic effect for monocytes of RPS19 dimers (73-76). Known partner proteins are FGF-2 (77), a fibroblast growth factor, which is involved in cell differentiation and migration, and Pim-1 (78), an oncoprotein. Pim-1 is a serine threonine kinase, which can indirectly regulate STAT5 activation and may be therefore involved in the signalling pathway of Epo-R. The recent demonstration of Pim-1 and RPS19 interaction is a major step towards the specific implication of RPS19 in the erythroid-signalling pathway.

Figure 3: rRNA maturation processing

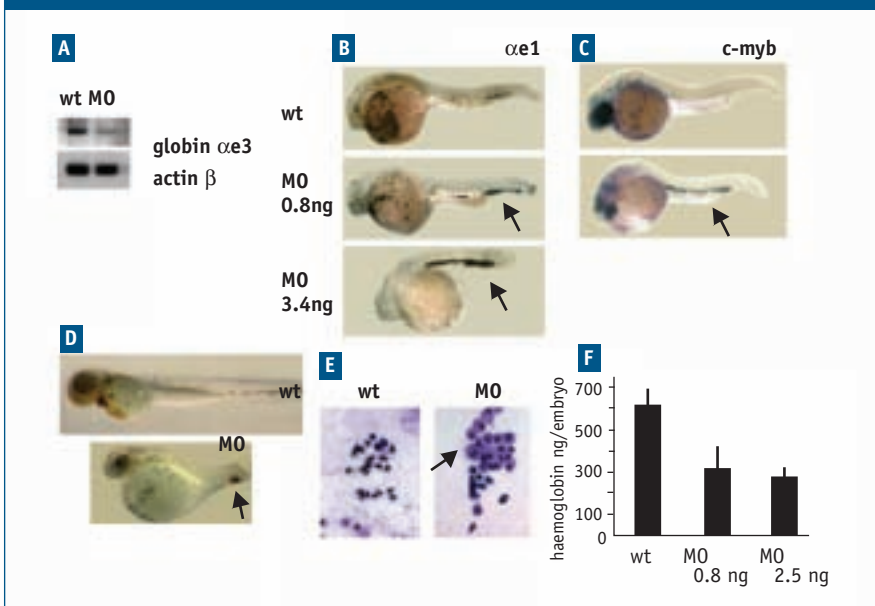


A. The normal rRNA processing of the 90S up to the mature rRNA 18S, 5.8S, and the 28S (25S in the yeast). **B.** The anomaly of the rRNA18S maturation in DBA: absence of the A2 cleavage in the 3'UTR of the internal transcribed space 1 (ITS1). After Léger-Silvestre et al., *J Biol Chem* 2005; 280: 38177-38185.

Until recently, animal models have been disappointing. In the fruit fly, *Drosophila melanogaster*, malformations such as delay in larval development, anomalies of bristles, wings or abdominal segments, short stature or infertility have been extensively described as the Minute syndrome (79-81). Minute syndromes have been shown to be related to mutations in a notable number of genes coding for ribosomal proteins. The mode of inheritance is dominant and homozygosity is lethal.

Haploinsufficiency has been clearly demonstrated, as has a correlation between its degree and that of phenotypic expression. However, there is no spontaneous Minute syndrome originating from mutations in the gene equivalent to human *RPS19*, which is located on the X chromosome. Manipulations of the promoter region with a P-element have resulted in a Minute-like syndrome, which can only be obtained in homozygous females or hemizygous males (Jason Fixler, personal communication). A phenotype in heterozygous animals has not been found using the murine model of *RPS19* gene inactivation, while early lethality of the homozygous blastocysts occurs (82). Recently, three animal models have been generated and appear to be very promising: a morpholino of *RPS19* in zebrafish (83) (Figure 4), Dark skin *Dsk3^{+/-}* mice, which corresponds to an amino acid change in *RPS19* (84) and a transgenic mice model, which reproduces the “hot spot” mutation, often described in DBA, p.Arg62Trp (85). This latter model exhibits growth retardation and anaemia

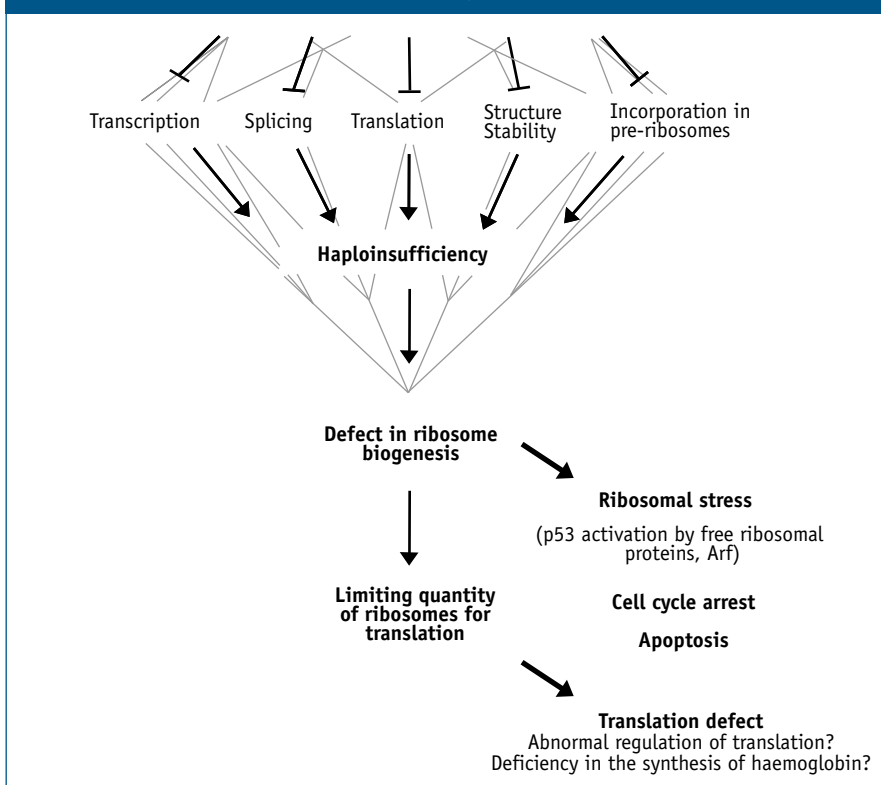
Figure 4: *RPS19* deficient zebrafish embryos



A. RT-PCR of erythroid progenitors from zebrafish embryos. Globin expression is lower in morphants. **B. C.** In situ hybridization globin *ae1* and *c-myb* probes respectively. Erythroid differentiation is delayed. **D.** Morphant erythroid cells are localised mostly in the posterior region (arrow) and only few cells are in the heart region. **E.** Morphant erythroid cells look like more immature than wild type ones. **F.** Erythroid differentiation is delayed in the morphant embryos with a decrease in haemoglobin concentration. After Danilova et al., *Blood* 2008; 112: 5228-5237.

associated with decreased erythroid progenitors in the liver and decreased BFU-e and CFU-e proliferation. Interestingly, both zebrafish and *Dsk3^{+/-}* animal models point out the activation of p53 pathway in RPS19 deficiency, which may be one important clue in the mechanistic understanding of DBA pathophysiology. Indeed, rRNA anomaly induces a nucleolar or a ribosomal stress. Free RPL5 and RPL11 bind MDM-2, an E3 ubiquitin ligase. p53, free from its natural inhibitor, MDM-2, is not degraded, but stabilised and activated (86, 87). It can thus play its role of the guardian of the genome, stopping the cell cycle and/or inducing apoptosis, which may only appear as the consequence of the p53 activation pathway. It is an attractive hypothesis, which needs to be confirmed but may explain the hypoplastic anaemia and apoptosis, both features, which are encountered in DBA (Figure 5).

Figure 5: Mutation of a ribosomal protein gene



Physiopathological hypotheses by Almass-Houd et al. M/S, accepted in October 2008

Strikingly, in addition to DBA, other bone marrow failure syndromes, namely cartilage hair hypoplasia, Shwachman-Diamond, and dyskeratosis congenita are also related to rRNA maturation impairment at different levels (88). All these diseases involved genes implied in the rRNA maturation, encoding for a nucleolar protein, reinforcing their role in ribosome biogenesis. DBA is thus a disease amongst other “ribosomopathies”. Recently, a ribosomal protein, RPS14 was described as responsible for an acquired haematological syndrome, the minus 5q syndrome (89). It is an acquired myelodysplastic syndrome characterised by anaemia, usually macrocytosis, thrombocytosis and myelodysplasia in the bone marrow, with predominance on the megakaryocytes. Fascinating advances in DBA research have been made over the last decade. Yet the gap between such advances and clinical and therapeutic relevance has been disappointing and may cause patients anguish and despair as they still have to face a chronic disease. It is our hope that the gap will be filled and that patients in the near future will benefit from the international collaborative effort on this orphan disease.

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Multiple Choice Questionnaire

To find the correct answer, go to <http://www.esh.org/iron-handbook2009answers.htm>

1. Diamond-Blackfan anaemia is:

- a) An acquired erythroblastopenia
- b) A congenital erythroblastopenia
- c) A sideroblastic anaemia
- d) A mitochondrial cytopathy

- 2. Diamond-Blackfan anaemia is due (according to current knowledge) to mutations in genes involved in:**
- a) Jak2 pathway
 - b) Ribosome biogenesis
 - c) Cell adherence
 - d) Glucid metabolism
-
- 3. Diamond-Blackfan anaemia is commonly treated with:**
- a) Vitamin B12
 - b) Gleevec (imatinib)
 - c) Iron
 - d) Steroids
-
- 4. Diamond-Blackfan anaemia evolution is characterised by all of the following, except one:**
- a) Unpredictable
 - b) Periods of independence from any treatment
 - c) A similar high risk of malignancy as in Fanconi anaemia
 - d) A high risk of complications during pregnancy, requiring specific management
-
- 5. The silent phenotype in Diamond-Blackfan anaemia corresponds to all of the following situations, except one:**
- a) Member of the propositus' family who carries a mutation in *RPS19* gene
 - b) Member of the propositus' family, who carries a mutation in *RPS24* gene
 - c) Member of the propositus' family, who exhibits an elevated level of the erythrocyte adenosine desaminase (eADA)
 - d) Member of the propositus' family, who exhibits macrocytic anaemia