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Bone Marrow Transplantation

Elevated thrombopoietin levels and alterations in the sequence of its receptor, c-Mpl, in patients with Diamond-Blackfan anemia

In the study of the possible thrombopoietin (TPO)-c-Mpl pathway involvment in the pathogenesis of Diamond-Blackfan anemia, repeatedly increased serum TPO levels were identified in 7/14 patients and changes in c-mpl sequence in 3/14 patients. While elevated TPO levels can represent a compensatory mechanism for impaired erythropoiesis, c-Mpl mutations could influence the disease severity.

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Thrombopoietin (TPO) is the major stimulator of megakaryopoiesis and platelet production.[1] Besides its function in megakaryopoiesis, TPO and its receptor c-Mpl are also involved in the production of progenitors of other hematopoietic lineages.[2] TPO acts synergistically with erythropoietin, greatly expanding the number of erythroid progenitors *in vitro*, as well as in mice after myelosuppressive therapy.[3] Its plasma level is inversely correlated to the mass of megakaryocytes and platelets, which degrade TPO following its binding to c-Mpl.[1]

Diamond-Blackfan anemia (DBA) is a congenital red cell aplasia characterized by normochromic macrocytic anemia, reticulocytopenia, normocellular bone marrow with a selective deficiency of erythroid precursors, normal or slightly decreased leukocyte count, and normal or slightly increased platelet count.[4] To elucidate the possible role of the TPO-c-Mpl pathway in the pathogenesis of DBA we measured TPO serum levels and screened *c-mpl* for mutations in these DBA patients.

In 7/14 (50%) of DBA patients, serum TPO levels were repeatedly higher than in age-matched controls (Table 1). Two of three patients with a mutation in RPS19 also showed elevated TPO levels. Interestingly, all patients had normal or slightly changed platelet counts, indicating that the general TPO level control mechanism may be altered in DBA patients and/or the increase in the TPO level may represent a compensatory mechanism for the promotion of their impaired

Table 1. Characteristics of DBA patients with increased plasma TPO levels.

Patient (sex)	*Age (years)*	*Type of anemia (treatment)*	*Associated anomalies*	*Platelet count (×10⁹/L)*	*Plasma TPO (pg/mL)*	*Age-matched controls (pg/mL); Average±SD*	*Mutation in RPS19*	*Mutation in c-mpl*
CZ2 (M)	28	mild (S)	thenar hypoplasia	225	215; 221; 259	52±20.7 n = 22	no	no
CZ3 (F)	21	remission	kidney aplasia	174*	178; 199	52±20.7 n = 22	no	Leu524Leu (C1570T)ˣ Val556Phe (G1666T)
CZ7 (F)	14	severe (TD)	no	174	215; 252; 278	52±20.7 n = 22	G167A R56Q	no
CZ9 (F)	18	mild (S)	no	293	178; 211; 235	52±20.7 n = 22	Del(196-206) frameshift	no
CZ19 (M)	8	mild (S)	short stature	530*	209; 215	88.4±19.5 n = 7	no	no
CZ21 (M)	5	severe (TD)	no	290*	239; 245; 390	80.5±23,8 n = 7	no	Val114Met (G340A)#
CZ23 (F)	3	severe (TD)	no	139*	304; 338; 589	90.8±29.1 n = 9	no	no

*TPO levels in all of these patients were at least 6 standard deviations above the average level in age-matched controls. Individual c-mpl exons were amplified by polymerase chain reaction (PCR); amplicons were purified and used as templates for sequencing using an ABI310 Genetic Analyzer (Perkin Elmer). Nucleotide numbering: A in the first start codon is considered +1. TD: transfusion dependency; S: steroid dependency; *: increased platelet count in infant age; x: mutations in the same allele (proved by PCR cloning of the region spanning exons 11 and 12, and by sequencing); #: the same substitution found also in a patient with a normal level of TPO; SD: standard deviation.*

erythropoiesis. This conclusion is in a good agreement with the finding that TPO enhances bone marrow erythropoiesis in DBA patients in the presence of erythropoietin and stem cell factor and/or interleukin-3.[5] Alterations in TPO serum levels have been described in a number of hematological disorders, however, the majority of changes are consistent with a model of platelet-mediated degradation of TPO.[6] In this respect, the finding of elevated TPO levels in 50% of DBA patients with normal or slightly changed platelet counts seems to be novel. Screening for mutations in *c-mpl* was carried out in 14 patients at the genomic level (DNA isolated from peripheral blood mononuclear cells), and in three patients a sequence alteration was identified (21.4%). In two patients, the same nucleotide change was identified in exon 3 (G340A) leading to an amino acid substitution Val114Met. It was reported that the 340A allele might represent a polymorphism with a frequency of 0.04.[7] Our own control data confirm this finding with a frequency of 0.05 (5 heterozygotes in 50 controls). The relevance of the Val114Met substitution for the function of c-Mpl remains to be determined.

In the third patient, two novel point mutations were identified in the same allele (and were also present in buccal swab and nails). The first mutation in exon 11 (C1570T) is silent (Leu524Leu), changing the wild type codon CUG to UUG. Although the mutation is silent, the wild type codon CUG is more than three times as frequent in human mRNA as the UUG codon (*Codon Usage Database; available at URL: www.kazusa.or.jp/codon*), raising the possibility that the level of c-Mpl protein may be influenced. The second mutation is located in exon 12 (G1666T), leading to a Val556Phe substitution. No mutation in either site was identified in 50 healthy controls, indicating that the 556Val may be significant for the c-Mpl function (556Val is located between box 1 and box 2 in the cytoplasmic domain of the receptor important for its internalization).[8] All affected amino acid residues are conserved in mice. Functional studies should reveal the effect of each single mutation on function or protein level of c-Mpl.

Homozygous mutations in *c-Mpl* have been reported to be the cause of congenital amegakaryocytic thrombocytopenia (CAMT) – a disease characterized by a severe hypomegakaryocytic thrombocytopenia with high levels of TPO which develops into pancytopenia in later childhood, suggesting a general defect in hematopoiesis.[9,10] Since a single normal allele of *c-Mpl* is sufficient for both normal megakaryopoiesis and erythropoiesis,[9,10] c-Mpl mutations observed in our patients are unlikely to be the primary cause of DBA. On the other hand, erythropoiesis in DBA patients is quite inefficient, so it is conceivable that additional changes in hematopoietic regulatory mechanisms could greatly influence the final output of red cells in these patients. Because TPO and *c-Mpl* seem to be essential for the production and maintenance of multipotent hematopoietic progenitors,[2] we conclude that (i) elevated TPO serum levels in DBA patients may represent a physiological compensatory mechanism to boost their impaired erythropoiesis; and (ii) alterations in the c-Mpl sequence could affect the course of the disease and/or influence the erythropoietic defect in DBA patients. To provide solid correlations between serum TPO levels, changes in c-Mpl and various DBA characteristics, studies with a larger cohort of patients are needed.

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Myelodysplastic Syndromes

Latency of onset of *de novo* myelodysplastic syndromes

The latency of onset of *de novo* myelodysplastic syndromes (MDS) is unknown. We report a retrospective analysis of blood counts from patients with MDS and acute myeloid leukemia (AML), and demonstrate temporal differences in rates of change of haemoglobin concentration and mean cell volume within 2-3 years of diagnosis, indicative of the earliest evidence of disease.

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The myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic stem cell diseases, whose aetiology is largely unknown, except for the relatively few cases presenting after exposure to cytotoxic chemotherapy.[1] Definition of the latency of onset of *de novo* MDS and acute myeloid leukemia may inform future epidemiological studies of the etiology of these diseases.

We have collected all known blood counts from 109 patients with MDS and AML over a 20-year period prior to the date of their diagnosis (all diagnosed after 1995). Sources of data were hospital case notes, computer databases, and community general practice notes. Co-existent