Defects in DBA: more than meets the eye

Lydie Da Costa

In this issue of Blood, Garçon et al describe successful derivation of induced pluripotent stem cells (iPSs) from fibroblasts of Diamond Blackfan anemia (DBA) patients with 2 distinct ribosomal defects. Using these cells, the authors showed that they not only exhibit defective erythropoiesis but also globally impaired hematopoiesis affecting multipotent progenitors.1

DBA is the first human ribosomopathy described. Mutations (among them large deletions in genes encoding a number of ribosomal proteins that include RPS19, RPL5, RPL11, RPS10, RPS26, RPS21, RPL35α, RPS17, RPS7, RPL36) have been identified in 70% of affected DBA patients.2 Haploinsufficiency of these ribosomal proteins results in distinct ribosomal RNA maturation defects, which in turn generates a nucleolar stress leading to the activation of p53 pathway. Indeed, activation of p53 has previously been shown to result in increased apoptosis and cell-cycle arrest in G0/G1 of erythroid cells in DBA accounting for the aregenerative anemia in the peripheral blood of patients.3,4 It should, however, be noted that pathways other than p53 activation could also play a role in defects in DBA.3

A major challenge in defining the mechanistic basis for hematopoietic defects in DBA has been the limited access to primary hematopoietic stem cells of DBA individuals. Previous attempts to generate iPSC cells using fibroblasts of DBA individuals have met with little success possibly due to activation of p53. The successful derivation of 2 clones of IPSs, 1 with mutant RPS19 and another with mutant RPL5 from DBA affected DBA patients by Garçon et al, is thus a major accomplishment and has the potential to open new avenues of research toward our understanding of this hematologic disorder.

The prevailing concept is that the hematopoietic defect in DBA affects primarily erythroid lineage and that the erythroid defect occurs at the progenitor stages between burst-forming unit-erythroid erythropoietin (EPO)-independent and colony-forming unit-erythroid EPO-dependent stages. For this reason, significant efforts in DBA research were focused on identifying causative mutations in erythroid genes such as EPO and EPO-R, SCF and c-kit, GATA-1. Thus, it came as a big surprise in 1999 when mutations in the gene-encoding ribosomal protein, RPS19, were first identified in association with DBA. In fact, there was considerable skepticism about the relevance of this finding because it was difficult to explain how mutations in a gene encoding a ribosomal protein result predominantly in anemia. Now that mutations in a large number of ribosomal proteins have been documented to account for DBA phenotype in >70% of cases, it is clear that DBA is indeed a ribosomopathy.

What are the implications of the reported findings? The findings of Garçon et al clearly show that haploinsufficiency of RPS19 and RPL5 results in defective erythroid differentiation of hematopoietic stem cells derived from IPS cells carrying ribosomal protein mutations (see figure). Importantly, rescue of the phenotype by expression of wild-type RPS19 and RPL5 in IPS cells validates that these mutations are indeed causative of defective erythropoiesis in DBA. Furthermore, their findings that ribosomal protein defects are pleotropic, affecting multiple hematopoietic lineages, is indeed exciting and implies we have to broaden our views on the role of ribosome biogenesis on all aspects of hematopoiesis. It is important to note that such pleotropic effects were suggested by the earlier work of Giri et al,6 Santucci et al,7 and Casadevall et al9 but these efforts have not received the recognition that they deserve.

The present findings also challenge the current perception that primitive and fetal erythropoiesis are not affected in DBA because most of the cases are diagnosed during infancy with only 16% of DBA cases diagnosed at birth and in the neonatal period. It is very likely that, in some instances, the DBA phenotype is expressed during fetal
development as indicated by the recent identification of a mutation in the \textit{RPS19} gene in a case of hydrops fetalis along with other cases of hydrops fetalis in DBA families.

The present study by Garçon and colleagues represents the first step in what should prove to be a long and productive journey toward a comprehensive understanding of the role of ribosomes in normal and disordered hematopoiesis and in evaluating new therapeutic options for the treatment of DBA.

\textbf{Conflict-of-interest disclosure:} The author declares no competing financial interests. □

\section*{REFERENCES}

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\section*{IMMUNOBIOLOGY}

\subsection*{Comment on Gertner-Dardenne et al page 922}

\subsection*{Inhibiting inhibitory pathways in human γδ T cells}

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In this issue of \textit{Blood}, Gertner-Dardenne et al show that B- and T-lymphocyte attenuator (BTLA) functions as an inhibitory receptor on γδ T cells and suggest that disruption of this inhibitory pathway in tumor-reactive γδ T cells may result in enhanced antitumor responses.1,2

Unlike αβ T cells, which recognize specific peptide antigens presented by major histocompatibility complex molecules, γδ T cells in contrast appear to recognize generic determinants expressed by cells that have become dysregulated as a result of either malignant transformation or viral infection. It is now generally accepted that as a result of dysregulation, tumor cells may display a variety of antigens that, although neither tumor-specific nor tumor-derived per se, can nonetheless serve as recognition determinants for cytolytic γδ T cells.3,5 It is this innate ability to recognize and kill a broad spectrum of tumor cell types—in a manner that does not require the existence of bona fide tumor-specific antigens—that has made the study of γδ T cells particularly appealing in the context of developing novel cell-based therapies directed against a variety of human cancers.6

Critical to any strategy intended to exploit either adaptive or innate antitumor immune responses is the development of an understanding of the mechanisms by which tumor cells can subvert, evade, or reshape such otherwise protective antitumor immune responses. To highlight this particular point, one need only consider the recent reports showing that antibody blockade of programmed death 1 (PD-1) inhibitory receptor pathways can lead to clinical responses in patients with a wide variety of tumors.7-8

BTLA is a more recently described inhibitory receptor that shares both structural and functional similarities with PD-1 and cytotoxic T lymphocyte–associated antigen 4 (CTLA-4).2 Although the function of BTLA as an inhibitory receptor on conventional αβ T cells is well established,9 its role in the regulation of human γδ T cells has remained unrecognized until now.

In the report by Gertner-Dardenne et al published in this issue of \textit{Blood},1 the authors establish that, through its interaction with herpes virus entry mediator (HVEM), BTLA expressed on human γδ T cells can moderate the activation, proliferation, and differentiation of Vγ9Vδ2 T cells, the predominant γδ T–cell subset found in human peripheral blood. Taken as a whole, this report provides evidence in support of a model in which the BTLA–HVEM interaction must now be counted as an important—and potentially targetable—inhibitory pathway that should be considered in the design of strategies intended to exploit the innate antitumor properties of human γδ T cells.

The authors first establish that BTLA and the T-cell receptor (TCR) colocalize within Vγ9Vδ2 T cells after mitogen stimulation. Importantly, this physical association was shown to negatively regulate TCR-mediated signaling because stimulation of Vγ9Vδ2 T cells through the TCR in the presence of blocking antibodies to BTLA resulted in increased phosphorylation of TCR-associated signaling molecules. Moreover, when Vγ9Vδ2 T cells were stimulated in the presence of HVEM-Ig (a soluble fusion protein that functionally engages BTLA), a significant reduction in Vγ9Vδ2 T-cell proliferation was observed. Conversely, when Vγ9Vδ2 T cells were stimulated in the presence of blocking antibodies directed against either BTLA or HVEM, substantially increased Vγ9Vδ2 T-cell proliferation occurred.

The authors conclude their report by presenting results obtained using more clinically relevant models. First, Vγ9Vδ2 T cells from healthy donors were stimulated in the presence of human lymphoma cell lines...
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