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the Tcl1 model. By using adoptive transfer approaches, the team also demonstrated that CD1d expression by CLL cells was not required for initial tumor control, implicating a third player in CD1d-dependent tumor surveillance by NKT cells.

Gorini et al then explored these findings with a series of experiments using samples from patients with stable and progressive CLL, confirming previous reports that high CD1d expression was more evident in the group with progressive CLL. NKT cell function was impaired in patients with progressive CLL, with poor responsiveness to stimulation with strong agonists, such as CD3/CD28 or phorbol myristic acid/ionomycin. This indicated that strong CD1d stimulation rendered NKT cells unresponsive to additional stimulation. Interestingly, these effects were observed in the absence of any exogenous CD1d ligand, demonstrating that a CLL self-ligand could be driving NKT cell exhaustion. Although the nuances of NKT cell anergy versus exhaustion were not explored due to a lack of material, these observations suggest that additional studies could be done to functionally characterize the "exhausted" NKT cells. Such characterization might also involve sublineage determination and whether there is any skewing away from an antitumor Th1 phenotype.

The authors then tested the hypothesis that NKT cells could suppress the CLL-supporting functions of monocyte-derived $CD1d^+$ nurse-like cells (NLCs). T cell–depleted peripheral blood mononuclear cells (containing $CD1d^+$ monocytes and $CD1d^{lo}$ or $CD1d^{hi}$ CLL cells) were cultured with healthy donor NKT cells. It was demonstrated that under conditions of low CD1d expression by CLL cells, NLCs did not differentiate and CLL cells were reduced in number. In contrast, cultures containing $CD1d^{hi}$ CLL cells resulted in higher numbers of differentiated NLCs and CLL cells.

Previously, one would understandably hypothesize that loss of CD1d expression by CLL cells could lead to evasion of NKT cell control. As a result of the new studies by Gorini and colleagues, a model was developed in which CLL evades NKT control indirectly. Under conditions of low CD1d expression by CLL cells, NKT cells are able to control the tumor via interaction with CD1d-expressing monocytes and NLCs, limiting their differentiation and ability to support CLL cells. However, under conditions of high CD1d expression, overstimulation (or at least chronic stimulation) leads to NKT cell exhaustion, such that they are unable to suppress NLC differentiation and subsequently support NLCs (see figure).

Although investigators and clinicians will await independent confirmation of these findings, this intriguing new model may have far-reaching implications for CLL diagnosis and treatment and may also impact research outside this immediate field. Firstly, CLL may remain a very useful diagnostic marker for early-stage and stable CLL. Furthermore, the use of exogenous CD1d-binding glycolipids to boost NKT cell control of CLL, as suggested in previous studies,[3] could be problematic. Under certain conditions, CD1d-binding glycolipids, such as α-galactosylceramide, render NKT cells anergic.[6] If such glycolipids are used for therapy, they could lead to desuppression of NLCs and have the unintended consequence of leading to more aggressive CLL.

A functional link between CD1d expression by B-lineage cells and NKT cells is also contributory to the humoral immune response to vaccination,[7] to blood-group antigen alloreactivity,[8] and to NKT cell–regulated autoreactive B cells in systemic lupus erythematosus.[9] Investigators in these other fields may wish to consider the consequences of low versus high CD1d expression by B-lineage cells and indirect mechanisms involving other $CD1d^+$ cell types.

The study by Gorini et al therefore sheds important new light on the mechanisms underlying the progression of CLL and may impact other areas of study. Arguably, new therapeutics for CLL may involve breaking the cycle between $CD1d^{hi}$ CLL cells, NKT cells, and NLCs.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● PLATELETS AND THROMBOPOIESIS

Comment on Vo et al, page 3486

Singling out FLI1 in Paris-Trousseau syndrome

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In this issue of *Blood*, using induced pluripotent stem cell (iPSC)–derived human megakaryocytes (iMegs), Vo et al find that the megakaryocyte/platelet defects observed in patients with Paris-Trousseau syndrome (PTS) are due solely to hemizygous deletion of the transcription factor (TF) FLI1.[1]

TFs bind to specific DNA sequences adjacent to the genes they regulate to promote or block the recruitment of RNA polymerase, thereby controlling the transcription of genetic information from DNA to RNA. Hematopoiesis critically depends on

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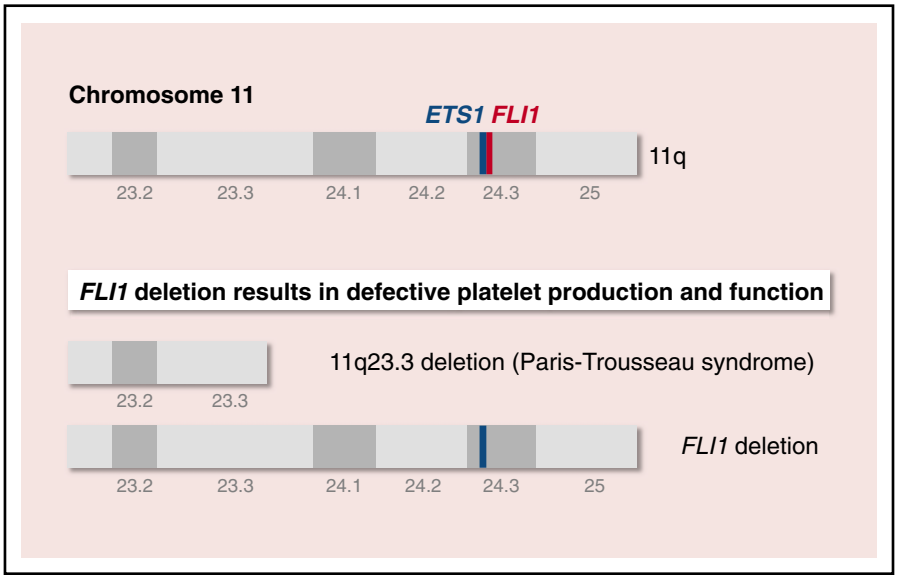

The genes encoding hematopoietic transcription factors ETS1 and FLI1 are located on chromosome 11q24.3, which is deleted in PTS. Using PTS-specific (11q23.3 deletion) and genome-edited (*FLI1* deletion) induced pluripotent stem cell–derived iMegs, Vo et al find that the megakaryocyte/platelet defects observed in patients with PTS are due solely to hemizygous deletion of the TF FLI1.

a complex network of TFs that temporally and spatially control the differentiation and maturation of individual lineages.[2] TFs work alone or in complexes. For example, in megakaryopoiesis, the TFs GATA1, FOG1, and RUNX1 form a complex with TFs of the E26 transformation-specific (ETS) family that includes ETS1, GABPA, and FLI1 to regulate megakaryocyte-specific gene expression. Mutations in TFs have been discovered in hematopoietic malignancies, and there is now increasing evidence that these mutations are an important underlying cause for defects in platelet production, morphology, and function.[3] These TFs include GATA1, RUNX1, FLI1, ETV6, and GFI1B.

Paris-Trousseau syndrome (Mendelian inheritance in man 188025) is a congenital platelet disorder encountered in most patients with Jacobsen syndrome (Mendelian inheritance in man 147791), a rare, inherited disorder characterized by skull dysmorphism, developmental delay, and multiple organ abnormalities. PTS is characterized by mild lifelong bleeding tendency, macrothrombocytopenia, bone marrow dysmegakaryopoiesis, and giant fused α-granules in a small population of platelets.[4,5] Genetically, PTS is due to terminal deletion on the long arm of chromosome 11, at 11q23.3, a region that contains the genes encoding ETS1 and FLI1, both located on chromosome 11q24.3 (see figure). Whether PTS is due to hemizygous deletion of 1 or both genes is unclear.

Based on the study of primary $CD34^+$ cells from patients with PTS, Raslova et al concluded in 2004 that the platelet disorder is due to allelic exclusion of FLI1 in $CD41^+/CD42^-$ progenitors, in which only the subpopulation of megakaryocytes originating from progenitors missing FLI1 are affected.[6] More recently, missense mutations and deletions in the DNA-binding domain of FLI1 have been implicated as the probable cause of the megakaryocyte/platelet defects observed in patients with PTS.[7,8] Although these reports suggest that FLI1 hemizygous deletion may be an important cause of the inherited thrombocytopenia, they did not detail the platelet defect or compare them with PTS platelets. Mice lacking FLI1 die of embryonic hemorrhage that is attributed to the role of FLI1 in both megakaryopoiesis and endothelium and hemangioblast specification.[9,10] However, hemizygous FLI1 deletion in mice does not recapitulate the human PTS phenotype.

To determine whether the megakaryocyte/platelet defects observed in patients with PTS were specifically the result of FLI1 hemizygous deletion and not to that of ETS1, Vo et al generated PTS-specific (11q23.3 deletion) and genome-edited (*FLI1* deletion) iPSC-derived iMegs and platelets (see figure). The study compares iMegs derived from a patient with PTS and iMegs in which FLI1 is hemizygously deleted ($FLI1^{+/-}$) using transcription activator-like effector nucleases. PTS and $FLI1^{+/-}$ iMegs replicate many of the described megakaryocyte/platelet features of PTS, including a decrease in iMeg yield and fewer platelets released per iMeg. Further, platelets from these FLI1-deficient human iMegs released in nonobese diabetic/severe combined immunodeficiency/interleukin-2 receptor γ-chain deficient (NSG) mice have poor half-lives and functionality, confirming the central role of FLI1 hemizygous deletion in the megakaryocyte and platelet defects observed in PTS.

Importantly, ETS1 is overexpressed in PTS and $FLI1^{+/-}$ iMegs, suggesting that FLI1 negatively regulates ETS1 in megakaryopoiesis and that the megakaryocyte/platelet defects observed in patients with PTS are unlikely to involve ETS1 allelic exclusion or deficiency, and are due solely to hemizygous deletion of FLI1.

Vo et al also overexpressed FLI1 in control and PTS iMegs using the glycoprotein Ibα promoter for megakaryocyte-specific expression and observed that FLI1 overexpression increases the yield of control iMegs in vitro and the yield, half-life, and functionality of released platelets in NSG mice, demonstrating that FLI1 overexpression enhances megakaryopoiesis, platelet production, and function.

In conclusion, the study by Vo et al confirms the central role of FLI1 hemizygous deletion in the megakaryocyte and platelet defects observed in PTS and demonstrates that FLI1 overexpression enhances megakaryopoiesis, thrombopoiesis, and platelet biology. Many questions remain unanswered; for example, whether FLI1 deletion affects the expression of other hematopoietic TFs such as GATA1, and whether ETS1 deletion reciprocally affects FLI1 expression remain to be addressed. Further, whether PTS and $FLI1^{+/-}$ iMegs exist as 2 subpopulations, because of allelic FLI1exclusion, as described by Raslova et al for $CD34^+$ derived PTS megakaryocytes,[6] is unclear.

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