

Multilineage dysplasia was detected in 32 cases (25%), a frequency similar to that reported by Falini, and was associated with a higher proportion of normal karyotype (93% vs 60%;  $P < .001$ ), lower leukocyte count at diagnosis ( $32 \times 10^9/L$  vs  $69 \times 10^9/L$ ;  $P = .01$ ), and lower bone marrow infiltration (51% vs 72% blast cells,  $P < .001$ ). Interestingly, the frequency of *NPM1* and *FLT3* internal tandem duplication (*FLT3*-ITD) mutations did not differ between patients with and without MLD (59% vs 50%, and 31% vs 38%, respectively). *NPM1* mutations were found in 68 patients (52%). MLD was observed in 19 patients (28%) with mutated *NPM1* and in 13 (21%) with wild-type *NPM1*. Outcomes in patients with mutated *NPM1* were similar for those with and without MLD; response rate was 95% and 85%, 5-year relapse incidence was  $35\% \pm 26\%$  and  $47\% \pm 16\%$ , and 5-year survival was  $56\% \pm 23\%$  and  $46\% \pm 14\%$ , respectively. In contrast in patients with wild-type *NPM1*, those patients with MLD showed an inferior response rate to induction chemotherapy (53% vs 85%;  $P = .02$ ). When the analysis was restricted to younger patients ( $\leq 60$  years) those with MLD showed a lower 5-year survival ( $0\%$  vs  $40\% \pm 16\%$ ,  $P = .012$ ; Figure 1). The unfavorable prognostic value of MLD on response rate ( $P = .034$ ; relative risk, 4.8; 95% confidence interval, 1.1-20) and survival ( $P = .036$ ; hazard ratio = 2.5; 95% confidence interval, 1.1-6) was confirmed in a multivariate analysis.

These results confirm that, although dysplastic features are a common trait in *NPM1*-mutated AML, they do not confer a worse prognosis. Falini et al found that gene expression profiling did not identify any distinctive MLD-associated gene signature in the mutated *NPM1* cohort.<sup>6</sup> The correlation found in the present study between an unfavorable outcome and dysplastic features in wild-type *NPM1* IR-AML patients leads us to suggest that a search for novel genetic or epigenetic markers in this AML subgroup might reveal a specific biologic identity.

In conclusion, the prognostic relevance of MLD in IR-AML might be dependent on *NPM1* mutational status. Whereas MLD predicts an adverse outcome in patients with wild-type *NPM1*, it lacks prognostic value in *NPM1*-mutated AML. Nonetheless, this observation requires further confirmation in a larger series of patients.

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**Contribution:** M.D.-B. updated the database of patients included in the analysis, performed all statistical analysis, and wrote the manuscript; M.R. performed morphologic review of all cases and reviewed the article; M.P. updated the database, performed molecular analysis, and reviewed the article; M.T. and M.C. performed molecular analysis and reviewed the article; J.L.I.A. performed morphologic review of all cases and reviewed the article; and J.E. designed the study, supervised statistical analysis, and reviewed the manuscript.

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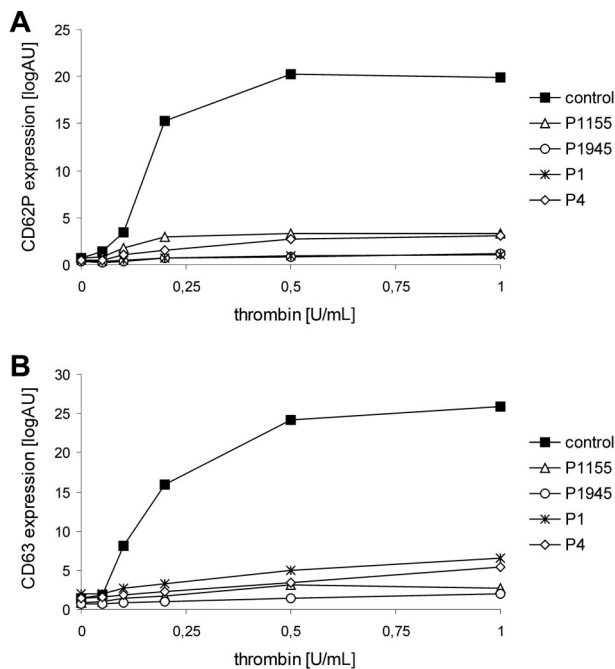
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## To the editor:

### Platelet secretion defect in patients with familial hemophagocytic lymphohistiocytosis type 5 (FHL-5)

Familial hemophagocytic lymphohistiocytosis (FHL) is a genetic disorder of lymphocyte cytotoxicity caused by mutations in the gene encoding perforin (FHL-2) or in genes encoding proteins

important for intracellular trafficking and exocytosis of perforin-containing lytic granules.<sup>1</sup> These include Munc13-4 (FHL-3), syntaxin 11 (FHL-4), and Munc18-2 (FHL-5). The molecular



**Figure 1. Thrombin-induced granule secretion of FHL-5 platelets.** Thrombin-mediated expression of CD62P (A) and CD63 (B) agonist on platelets was detected by flow cytometry. Diluted PRP ( $5 \times 10^7/\text{mL}$ ) was stimulated with different concentrations of thrombin (0.05–1.0 U/mL; Dade Behring) in the presence of 1.25mM Gly-Pro-Arg-Pro/GPRP. Platelets were stained by monoclonal anti-CD62P antibody (CLBthromb/6-FITC, Immunotech) and anti-CD63 antibody (CLB-gran/12-FITC), respectively. Data are expressed as median of logarithmic fluorescence intensities.

machinery for the transport of lytic granules in part overlaps with that required for the transport of lysosome-related organelles in other cellular systems. This is well illustrated by immunodeficiency syndromes associated with albinism such as Griscelli syndrome type II (GSII), Chediak-Higashi syndrome (CHS), and Hermansky-Pudlak syndrome type 2 (HPS2). The genes affected in these diseases (*RAB27A*, *LYST*, and *AP3B1*) are also relevant for hair pigmentation, neurologic development, neutrophil development, and platelet function leading to manifestations such as partial albinism, mental retardation, neutropenia, and bleeding in addition to a high risk of hemophagocytic lymphohistiocytosis (HLH).<sup>2,3</sup>

The recently described FHL-5 is considered to be mainly a disorder of cytotoxicity.<sup>4,5</sup> A role for Munc18-2 has been demonstrated in mast cell degranulation,<sup>6</sup> but a clinically relevant role for Munc18-2 deficiency in cell systems other than lymphocytes has not been documented. Munc18-2 is expressed in platelets and associates with a large complex containing synaptosome-associated protein of 23 kDa (SNAP-23) and cellubrevin/vesicle-associated membrane protein 3 (VAMP3).<sup>7</sup> These data suggest that Munc18-2 might play a role in platelet granule exocytosis. In support of this concept, it was recently reported that 3 of 11 FHL-5 patients developed significant bleeding symptoms even outside of acute HLH episodes.<sup>8</sup> However, all of these patients were also thrombocytopenic and platelet function tests were not performed.

Here we report on platelet function analysis of 4 patients with genetically confirmed FHL-5. The *MUNC18-2* mutations of these patients, their clinical course, and data showing impaired T cell and NK cell degranulation have been described previously<sup>4,9</sup>: patients P1155 and P1945<sup>4</sup> showed a typical course of FHL with manifestation of hemophagocytic syndrome

within the first year of life, while patients P1 and P4<sup>9</sup> presented with a late onset of FHL. Of note, none of the patients had obvious bleeding symptoms.

Flow cytometric analyses of the patients' platelets revealed that platelet  $\alpha$  (CD 62P)- and  $\delta$  (CD 63)-granule secretion in response to thrombin stimulation was severely impaired in all patients (Figure 1). Surface expression of glycoprotein (GP) Ib/V/IX and GPIIb/IIIa, ristocetin-induced binding of Von Willebrand factor, and binding of soluble fibrinogen were normal in all patients. Platelet aggregation/agglutination after stimulation with ADP, collagen, and ristocetin was slightly impaired. Bleeding time was assessed in P1945 and was slightly prolonged (8.5 minutes). These data demonstrate a selective impairment of platelet granule secretion in patients with FHL-5 and thus document an important role for Munc18-2 in platelet degranulation. Platelet secretion defects have also been observed in patients with HPS2 or with CHS who can present with mucocutaneous bleedings, especially after surgery.<sup>2,10</sup> Although bleeding symptoms in FHL-5 patients seem to be mild, our findings clearly demonstrate that Munc18-2 deficiency is more than a genetic disorder of cytotoxicity.

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## To the editor:

### Platelet interior imaging technologies

Harry F. G. Heijnen and his colleagues recently reported studies of the platelet interior using electron tomography.<sup>1</sup> The methods used to freeze platelets and prepare them for cryo-electron tomography are of interest. However, most of the information presented is not new. A major finding was that the open canalicular system and dense tubular systems of channels were highly intertwined and formed close associations in specialized membrane regions. This observation is not new. Interaction of the 2 channel systems was first reported in 1972,<sup>2</sup> and the name “membrane complexes” assigned to them. Their similarity to the sarcoplasmic reticulum of embryonic muscle and ability of the dense tubular system to bind divalent cations suggested a role of membrane complexes in platelet muscle physiology.<sup>3</sup>

A review chapter, “Platelet Structure,” at page 70 (Figures 3-74, 3-75, and 3-76) in the second edition of Michelson’s textbook *Platelets*<sup>4</sup> provides a more comprehensive picture of membrane complexes. Identification of  $\alpha$  granule subtypes included organelles containing the multimeric von Willebrand factor assemblies in tubules eccentrically located in the  $\alpha$  granule matrix.<sup>1</sup> Heijnen and colleagues cite a paper by Cramer et al<sup>5</sup> for recognizing the tubular elements in  $\alpha$  granules, demonstrating that they are von Willebrand factor multimers similar to Weibel-Palade bodies. However, an earlier study in 1968<sup>6</sup> described them in thin platelet sections by transmission electron microscopy (TEM). The authors of the current paper<sup>1</sup> frequently encountered  $\alpha$  granules with an elongated, “tubular shape,” and their Figures 4 and 5 provide images of the tubular subtypes. However, images in Figure 3-49 on

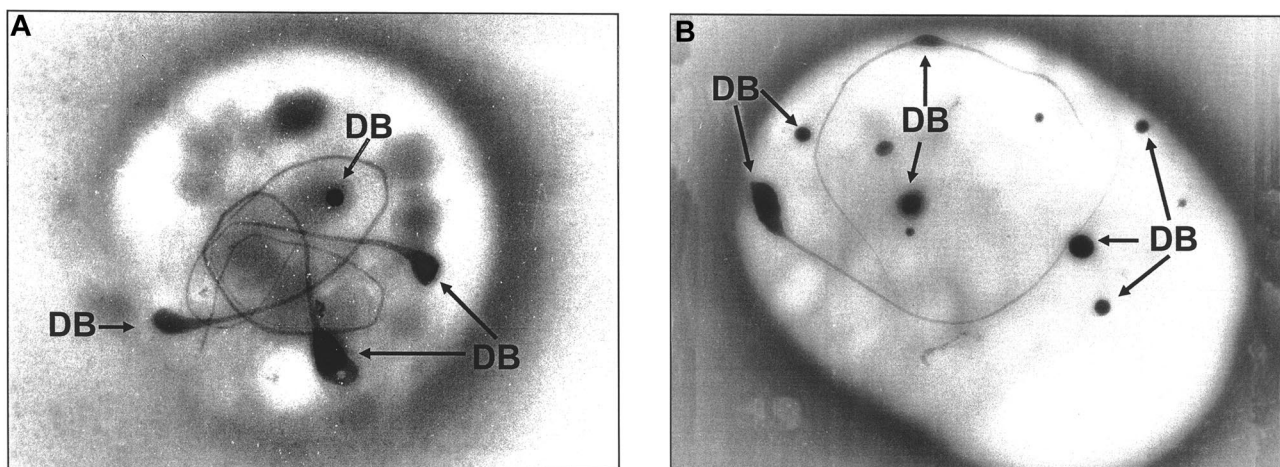
page 62 of Michelson’s text<sup>4</sup> provide an image of  $\alpha$  granules with extensions taken by TEM that is clearer than provided by tomography. The extensions appear as dense as the granules from which they originate. Therefore, we referred to them as “rod-like,” rather than “tubular.” The presence of cross-striations in the rods also was noted in another study.<sup>7</sup>

Electron tomography or its interpretation may make errors in organelle identification. The tomographic slices of a “tubular”  $\alpha$  granule in Figure 5C-F and its reconstruction in 5G-H are interesting, but the organelle is not an  $\alpha$  granule. It is a  $\delta$  granule (dense body). Several examples are shown in Michelson<sup>4</sup> on page 62 in Figures 3-50 and 3-51, on page 63 in Figure 3-52, and in examples included here (Figure 1). The images in the enclosed illustrations were taken by TEM on whole-mount preparations of normal platelets.

In summary, Heijnen et al have used a useful new technology to review the platelet interior. However, based on the concerns raised in this letter, it does not appear that electron tomography has replaced thin-section and whole-mount TEM.

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**Figure 1. Whole-mounted platelets viewed by TEM.** Two micrographs (A-B) of whole-mounted platelets viewed by TEM contain many spherical dense bodies (DBs) and DBs with 1 or 2 tails.