are unlikely to have differentially identified new cases that previously would have been missed. Further, ethnic differences in incidence trends have only been observed in childhood ALL and are not present in other childhood cancers (eg, childhood brain tumors), suggesting that changes in ethnic classification are unlikely to be solely responsible for trend differences. In utero exposure to factors such as tobacco are likely to have remained stable or decreased in prevalence during this period.\(^3,4\) Other suspected risk factors for childhood leukemia that may explain the increase in incidence in Hispanic children, such as childhood atopic conditions, pesticide exposure, and maternal and child weight, have increased during this period, but may not be specific to Hispanic children and require further evaluation. Large, well-designed epidemiologic studies of childhood leukemia with diverse ethnic backgrounds, such as the California Childhood Leukemia Study, seek to further elucidate the reasons for the observed increases in incidence rates among Hispanic children in the last 2 decades.

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To the editor:

**Platelet dense granule secretion defects may obscure α-granule secretion mechanisms: evidence from Unc13d-Jinx deficient platelets**

We were very interested to read in Blood the recent papers by Meng et al, “Defective release of α-granule and lysosome content from platelets in mouse Hermansky-Pudlak syndrome models” and by Sharda et al, “Defective PDI release from platelets and endothelial cells impairs thrombus formation in Hermansky-Pudlak syndrome.”\(^1,2\) In these papers, the authors show that platelet adenosine diphosphate (ADP) secretion from dense granules is an important autocrine regulator of α-granule secretion. We have reached essentially the same conclusion, however, from a different perspective. We believe that, together, our data show that experimental manipulations or conditions that disrupt dense granule secretion may indirectly affect α-granule secretion and should be taken into account when investigating the mechanisms of α-granule secretion.

We are interested in the molecular machinery that controls platelet granule secretion. The SNARE regulator, Munc13-4, was shown to be an essential factor for dense granule secretion.\(^3\) Consistent with this, Unc13d-Jinx mice (which lack Munc13-4) have prolonged tail bleeding and are protected from arterial thrombosis and cerebral infarct progression.\(^3,5\) It has also been previously shown that Unc13d-Jinx platelets have a substantial, but incomplete, suppression of α-granule secretion,\(^3\) an effect that we also observe. Washed platelets were stimulated with PAR4-activating peptide (PAR4-AP) (300 μM) and surface expression of CD62P was used as a marker of α-granule secretion. PAR4-AP–induced CD62P expression was significantly less in Unc13d-Jinx platelets compared with wild-type (WT) platelets (Figure 1A). This suggests that almost all α-granule secretion is dependent on Munc13-4. However, the block to α-granule secretion in the absence of functional Munc13-4 was substantially rescued by the addition of ADP (Figure 1A-B). Therefore, there must also be a Munc13-4–independent pathway that is capable of inducing substantial α-granule secretion.

ADP alone was not able to induce detectable CD62P expression under these conditions, suggesting that ADP acts synergistically to enhance PAR4-AP–induced α-granule secretion. In the presence of the P2Y12 antagonist AR-C69931MX, there was a marked suppression of CD62P surface expression in both WT and Unc13d-Jinx platelets, and the ADP-mediated rescue of secretion was ablated (Figure 1B). The P2Y1 antagonist, MRS2279, had no effect. In addition, we found that 5-HT was not able to induce α-granule secretion, and
neither did it synergise with PAR4-AP to rescue the response in the Unc13dJinx platelets (data not shown), suggesting that 5-HT has no role in the regulation of secretion under these conditions. Therefore, secreted ADP, acting through the P2Y12 receptor, synergizes with PAR4-AP to induce Munc13-4–independent α-granule secretion, but the presence of this pathway is obscured by the complete loss of dense granule secretion in Unc13dJinx platelets.

To confirm that secreted ADP regulates PAR4-AP–induced α-granule secretion, we also investigated the role of ADP in the release of the α-granule cargo, PF4 (Figure 1C). PAR4-AP–stimulated Unc13dJinx platelets released significantly less PF4 than WT platelets. Co-stimulation with ADP induced more PF4 release in Unc13dJinx platelets than PAR4-AP alone (34.3 ± 2.2% of total PF4 with PAR4-AP+ADP compared with 19.0 ± 2.3% with PAR4-AP alone), again suggesting that loss of secreted ADP contributes to the α-granule secretion defect in these platelets.

These data demonstrate that the disruption of dense granule secretion can alter the apparent contribution of different proteins to α-granule secretion. For example, under our stimulation conditions, we would have concluded that Munc13-4 is a major regulator of PF4 release, whereas it may be released in a largely Munc13-4–independent manner that is enhanced by secreted ADP. There was still a small, statistically significant, difference between PF4 release from WT and Unc13dJinx platelets in the presence of ADP, suggesting that there may be a small direct contribution of Munc13-4 under these conditions. Alternatively, this may reflect a small contribution of other dense granule components, or that exogenous ADP does not fully mimic locally high concentrations of secreted ADP. The relative contribution of the Munc13-4–dependent pathway and the Munc13-4–independent pathway may depend on the precise conditions of platelet stimulation. However, we believe that our data raise the more general question: How much do we know about the molecular mechanisms of α-granule release in platelets? It will be particularly interesting to re-evaluate knockout mice studies that show defects in dense and α-granule secretion, to determine which proteins regulate α-granule secretion directly, and which act indirectly through dense granule secretion.

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References


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