Wide Variation in Platelet Ganglioside Content in Individual Platelet Donors

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Introduction

Glycosphingolipids (GSLs) are a heterogenous family of biomolecules, containing a carbohydrate head group and ceramide (Cer) lipid tail. Human platelets express predominantly globo- and neolacto-family GSLs, which have been shown to play a role in infection, PLT activation, and the PLT storage lesion. GM3 is the major sialylated GSL or ganglioside on human PLTs and is critical for megakaryocyte differentiation. GM3 may also play a role in cold storage, membrane reorganization and cell signaling via glycolipid-enriched microdomains. GM3 is a related pro-apoptotic ganglioside that is reportedly increased with PLT activation and PLT storage. We have previously shown significant donor-specific differences in neutral GSL expression in individual PLT donors. We have now screened the ganglioside fraction from individual donors for donor-specific differences in GM3 and GD3 expression. The latter could represent uncharacterized donor factors influencing PLT viability and recovery following in vitro storage.

Methods

PLTs from 127 outdated 6-7 day old single donor apheresis units were isolated, washed, lyophylized, and then extracted with 20 vol chloroform-methanol (C-M 1:1) for 72 hours. The total lipid fraction was separated into neutral and acidic lipid fractions by DEAE column chromatography, followed by saponification and dialysis to hydrolyze contaminating phospholipids. The acidic lipid dialysate was dried, resuspended in C-M 85:15, applied to a silicic acid column and washed with 20 volumes C-M 85:15 to remove sulfatides & phospholipid contaminants. Gangliosides were isolated by eluting the column with C-M 1:2. Total ganglioside yield was determined using the resorcinol-HCl assay and reported as lipid-bound sialic acid (nmoles LBSA) per unit and per 10^{10} PLTs. Individual gangliosides were analyzed by high performance thin layer chromatography (HPTLC) and scanning densitometry. Results were compared to PLT yield, neutral GSL expression, donor age, sex, ABO, HLA, CMV status and donation history. Statistics were performed with commercial software.

Results

Ganglioside GM3 Content in Individual PLT Donors

GM3 was the major PLT ganglioside, averaging 90% of the total ganglioside (Fig. 3A and 3B). Sialoparagloboside (SPG, 5%) and an array of related, HMW neolacto-gangliosides with sialyl-I, sLeX and ABO activity constituted the remaining ganglioside species. The calculated number of GM3 per PLT varied 500-fold between donors (15000-8537000/PLT; 2.38-8.4x10^{11}). This was particularly evident for group B units, which were collected on the Haemonetics MCS with lower PLT yields, LBSA content and GM3/PLT were constant (P=0.5 , paired t-test). There was no direct correlation between GM3 and neutral GSL expression (Fig 1); however, LBSA and GM3/PLT tended to be higher among CDH-rich (85% neutral GSL) versus globio-rich (GB3+GB4, 85% neutral GSL) donors (437000 versus 29000 GM3/PLT, P=0.04).

Platelet GD3 Content

Ganglioside GD3 is normally a minor PLT ganglioside although GD3 is reportedly increased with PLT activation and storage, where it may play a role in mitochondrial depolarization. In most donors, GD3 was <1% total ganglioside (Fig. 4, sample D-86). In 500 600

Lipid Bound Sialic Acid (LBSA) per Unit

The nmoles LBSA per unit ranged from 15.5 to 864.2 nmoles (Fig. 2A and Table 2). The LBSA was weakly correlated with PLT yield (Fig. 2B, R=0.41), which ranged from 2.38-8.4x10^{11}. This was particularly evident for group B units, which were collected on the Haemonetics MCS with lower PLT yields, LBSA and neutral GSL (mg) per unit. Overall, group A units tended to have significantly higher LBSA/unit, even after normalizing for PLT count (P=0.01-0.001; Table 2). There was also a trend toward higher LBSA among female donors (P=0.08).

Conclusions

GM3 concentrations vary significantly between PLT donors. With 1 exception, differences in GM3 did not reflect either desialylation (GM3→CDH) or CDH generation (GM3→GD3). The inverse association between globo-GSL expression, CDH and GM3 content resembles the action of golgi inhibitors, suggesting golgi regulation of megakaryocyte and PLT GSL synthesis. Donor-specific differences in GM3 and GD3 could represent donor factors effecting PLF function, viability and recovery following in vitro storage.