Expression of a LKE-Related Globo-Glycosphingolipid in Platelets is Dependent on Specific Platelet Glycotypes

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Introduction

E. coli-associated hemolytic uremic syndrome (HUS) is associated with the secretion of shiga toxins (Stx), which specifically recognize and bind terminal Galα1-4Gal epitopes present on membrane glycosphingolipids. The primary GSL receptor on PLTs,1-2 endothelium and renal epithelium is the Pα antigen or globotriaosylceramide (Gb3, Fig. 1). PLTs can also express a second receptor, Band 0.03. The latter is a globo-GSL related to Pα, P and LKE antigens.1 In a small study of individual PLT donors (n=25), Band 0.03 was identified in 20% (5/25).1 We now report the incidence and expression of Gb3 and Band 0.03 in a large population of PLT donors.

Fig. 1 GSL Receptors for Shiga Type Toxins in PLTs and RBC

Methods

Glycosphingolipid (GSL) Isolation

Outdated (6-7 days) PLT GSLs were isolated as described.1 Briefly, PLTs were extracted with chloroform-methanol (C:M 1:1, v/v) and then separated into neutral and acidic lipids by anion exchange chromatography. The neutral lipid fraction was dried, saponified with 0.3N methanolic NaOH, dialyzed and applied to a silicic acid column. The latter was sequentially washed with C, ethyl acetate, acetone-methanol 9:1 and 7:3. The last two fractions were pooled as the neutral GSL fraction.

GSL Analysis

Individual GSLs were separated by high performance thin layer chromatography (HPTLC) and visualized with diphenylamine reagent or by immunostaining with shiga toxin (Stx).1 GSLs were characterized by mobility (Rf) and relative area by scanning densitometry.

Results

Differences in Gb3 expression in Individual PLT Donors. GSLs were isolated from 127 apheresis PLT samples tested. There was a correlation between %Gb3 and Band 0.03 expression. Gb3 levels increased Gb3 and Band 0.03 expression. Gb3 levels were significantly higher in Band 0.03+ donors (33.9% vs 21.1%, P<0.0001). When examined by PLT glycotype, Band 0.03 was strongly linked to Type E and F (Fig. 7B).

Shiga Toxin (Stx) Binding is Highest in Two Globo-Rich PLT Glycotypes.

Donors were classified into 6 PLT glycotypes based on the distribution (%) of CDH, Gb3 and Gb4 (Figs. 4 & 5). The highest Stx-Gb3 binding was observed in Types E and F (Fig 6, area), which are rich in globo-GSLs (32-39% Gb3, Fig 5). In contrast, little or no binding was observed to Types A-C, which possess very little Gb3 (1-20%).

Fig. 4: HPTLC of PLT Glycotypes A-C (Diphenylamine)

Fig. 5. GSL Distribution (% GSL) by PLT Glycotype

Band 0.03 Associated with Specific PLT Glycotypes

Band 0.03 (Fig. 7A) was identified in 12/91 (13%) samples tested. There was a correlation between increased Gb3 and Band 0.03 expression. Gb3 levels were significantly higher in Band 0.03+ donors (33.9% vs 21.1%, P<0.0001). When examined by PLT glycotype, Band 0.03 was strongly linked to Type E and Type F (97%; X² P=0.00049). Among Band 0.03+ donors, 31% were Type E and 56% were Type F (Fig. 7B).

Platelets do not express Pₐ antigen

We previously reported that Pₐ antigen is not expressed by pooled human PLTs (Fig. 7A, band 0.06).1 We now report the absence of Pₐ in nearly 92 individual PLT donors.

Conclusions

In a large study of PLT donors, the incidence of band 0.03 was 13% and is slightly lower than previously reported (20%).1 Expression of band 0.03 was highly correlated with two PLT glycotypes (E,F). Individuals with these glycotypes may have an inherent increased susceptibility to Stx-induced PLT activation and HUS. In contrast, individuals with glycotypes A-C may be relatively resistant to Stx.