Supplementary Data

Technical development and validation of PDVF Glycoarray

By comparing results obtained from testing a prototypic anti-GM1 monoclonal antibody (DG2) (Townson et al. 2007) against duplicate spots of this ganglioside, and following optimisation of the printing protocols, washes, and incubation conditions, the inter- (n=5) and intra- (n=9) assay coefficients of variation were measured at 4.1% and 8.6%, respectively (supplementary figure A,B), comparable with ELISA (Kuijf et al. 2005; Willison et al. 1999). By adding 20% fluorescent BODIPY FL C_5-GM1 (Molecular Probes, UK) to the standard GM1 working solution, we were able to show that a 50:50 mixture with GD1a did not significantly ($p=0.55$, two sided two sample t-test, n=6) alter the amount of GM1 bound to the membrane as compared with a 50:50 mixture with methanol alone (supplementary figure C,D), as detected by measuring fluorescent intensity with a Storm PhosphorImager (GE Healthcare, UK). This result further suggests that any enhanced binding to GM1:GD1a complexes, as compared with GM1 alone, is not due to increased amounts of GM1 binding to the membrane in the presence of GD1a.

Combinatorial PDVF Glycoarray Protocol

GM1, asialo-GM1, GM2, GM3, GM4, GD1a, GD1b, GD3, GT1b, sphingomyelin, and cholesterol, were obtained form Sigma, UK. GT1a and GQ1b were obtained from Accurate Chemical and Scientific, USA, and GD2 from Calbiochem, USA. Stock solutions of each of the above were prepared in a 50:50 (v/v) chloroform:methanol mixture, at 1 to 10 mg/ml. Working solutions were made by further dilution in methanol to 0.1 mg/ml. For single samples, 200 µl of the working solution was added to a 300 µl capacity micro-sampling vial (Chromacol, UK). To create complexes, 100 µl of each constituent GSL was added to a vial, sealed using caps with a rubber insert (Chromacol, UK), and then sonicated for 3 minutes prior to use.

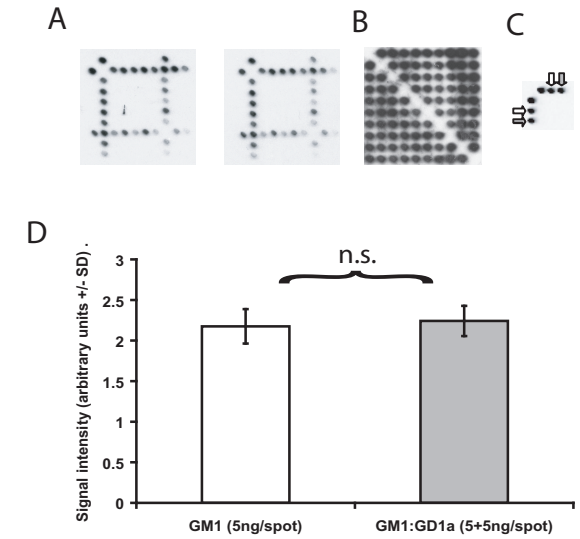
Sheets of PVDF membrane (Sigma, UK) were cut into 20x25 mm squares using a scalpel. These were then glued (UHU GmbH, Germany) 12 mm from the left hand edge of a plain glass slide (VWR International, UK), and allowed to air dry for 10 minutes. A metal grid was used to hold 12 slides in predefined positions on the application plate of a Camag Automatic TLC Sampler 4 (Camag, Switzerland). The winCATS planer chromatography management software (Camag, Switzerland) was used to write programmes which result in the application of duplicate spots of 0.1 µl of 100 µl/ml ganglioside or ganglioside complex over a predefined 0.4 μm^2 area. Printed membranes were outlined with a hydrophobic barrier pen (Vector Laboratories, UK) and allowed to air dry for 20 minutes. They were then stored overnight at 4ºC before use.

Membranes were blocked in at least 100 ml/cm^2 of 2% bovine serum albumin/phosphate buffer saline (BSA/PBS) for 1 h at 4ºC. Serum samples, CSF, monoclonal antibodies, siglec-Fc fusion proteins (preconjugated to horse radish peroxidase (HRP) linked anti-Fc antibody), or HRP-bacterial toxin conjugates (Deinhardt et al. 2006) were diluted in 1% BSA/PBS. 500 µl of this sample was then applied to a pre-printed membrane and incubated at 4ºC. After 2 h, the sample was tipped form the membrane and the slides were briefly placed back in the 2% BSA/PBS blocking solution. Probes requiring a secondary antibody underwent a primary wash phase. These membranes were transferred to at least 500 ml/cm^2 of 1% BSA/PBS for 15 minutes of washing on a shaker set at 100 rpm. This process was repeated once. These membranes were tapped dry, 500 µl of the appropriate HRP-linked secondary antibody was applied (diluted in 1% BSA/PBS, typically to 1:30,000), and incubated for 30 minutes at 4ºC. Membranes were then washed. For probes not requiring a secondary antibody (siglecs and HRP-conjugated bacterial toxins), this immediately followed the primary incubation.

Membrane wash was standardised into two changes of 1% BSA (30 minutes) and three changes of PBS (5 minutes), again each of at least 500 ml/cm^2, on a shaker set at 100 rpm. Slides were then briefly dipped in two changes of distilled water (500 ml/cm^2). Detection was then performed using ECL plus (Amersham/GE Healthcare, UK) using 450 µl of detection mix for 3 minutes at room temperature. The solution was tipped from membranes and signal was detected on radiographic film. Exposure time was 15 s. Films were digitised by flatbed scanning and the images quantified by ImageQuant TL software (Amersham Biosciences, UK). The average intensity value for the 10 negative control spots was subtracted from all other spots. The value for the most intense spot was then normalised to 100%. Minitab statistical software was used to detect significant differences between the relative signal intensity generated by binding to the single ganglioside (designated as the control level) as compared with 1:1 complexes containing that ganglioside. General linear model ANOVA with Dunnett's method was applied to correct for multiple comparisons. Comparisons between specific ganglioside complexes of each series and their component individual gangliosides were assessed by multiple two-sample t-tests with Bonferroni's correction. Normality was verified by the Kolmogorov-Smirnov test ($p>0.15$ indicating normality).

Supplementary Figure Legend

Supplementary Fig. Inter-assay variability was determined by assaying 5 different membranes with the anti-GM1 monoclonal antibody DG2 on different occasions, and measuring the intensity of the GM1 signal. Two example processed grids are shown (**A**). To determine intra-assay CV, nine separately prepared GM1 samples were spotted in duplicate around the edges of an array, and again probed with DG2 (**B**). The amount of GM1 remaining bound to the PVDF membrane following application of a 50:50 mixture of GM1 (containing 20% fluorescent GM1) and methanol (open arrows and bar) was not significantly different to that observed for a 50:50 mixture of GM1(with 20% fluorescent GM1) and GD1a (filled arrows and bar), as measured by fluorescent intensity detected by a phosphorimager (**C,D**). This counters the hypothesis that the modulated binding seen with mixtures of gangliosides is simply a result of alterations in the amount of ganglioside binding to the assay platform when complexes are applied.



Supplementary Figure