Title: Healthy Donor Red Blood Cells Express Unique Glycan Fingerprints

Authors: Anani W, Ashwood H, Schmidt A, Burns R, Denomme G, Hoffmeister K

Background/Case Studies: Lectins are glycan recognizing proteins, some of which can distinguish between ABO blood types. Lectin arrays provide an extensive set of glycan probes for rapid analysis of biological samples. Red blood cells contain a variety of surface glycans that, in combination, act a as unique signature. Our aim was to create a healthy donor red blood cell database of glycan fingerprints by ABO blood type and data mine unique glycan signatures attributed to the donors.

Study Design/Methods:

Cell Fraction Preparation: Donor EDTA whole blood were collected, centrifuged to obtain red blood cells, and further purified with Ficoll and dextran separations. Erythrocyte ghosts were prepared by lysis and membrane bound proteins were extracted with the Mem-Per Plus kit (ThermoFisher, Rockford, IL).
Lectin Array Analysis: Isolated membrane proteins were incubated with Cy3 monoreactive dye pack (GE Healthcare, Fairfield, Connecticut) and labeled protein was added to a glass lectin microarray slide with 45 printed lectins (LecChip, Glycotechnica). Fluorescent microarray images were acquired using an evanescent-field fluorescence scanner (GlycoLite 2200; Glycotechnica)
Data Analysis: All fluorescence data were analyzed using Signal Capture and GlycoStation Tools Pro (v1.5, Glycotechnica). The background subtracted, sum raw intensity of the lectin spots were quantile-normalized (R v3.5.2, Vienna, Austria) and plotted with intensity heat maps and principal component analysis. A linear model analysis was used to calculate p values between ABO blood types. Adjusted p values <0.5 were considered statistically significant.

Results/Findings: A total of 80 donor red blood cells (n=20 each ABO blood type) were analyzed. Blood types were statistically different based on different combinations of lectins: 24 lectins between O vs AB, 13 O vs A, 13 A vs AB, 13 O vs B, 9 B vs AB, and 4 A vs B (Table). Principal component analysis identified clusters of blood types with varying surface glycoprotein expression of ABO blood types. Principal components 1 and 2 accounted for 35% and 26% of the variability in each axis, respectively. DBA lectin was the strongest predictor of Group A; UEA-I, SBA, and BPL lectins for Group O; Calsepa, EEL, and LTL lectins for Group B; and DBA, EEL, Calsepa lectins for Group AB. When examined by lectin binding, some donors had weakened expression of their A or B blood type (4 group B donors, 2 group A donors) or were serologically Group O but weakly expressed another blood type (5 expressing group B, 1 expressing group A). Group B showed a wide variability of surface expression.

Conclusions: Lectin arrays can detect subtle surface ABO blood type glycoprotein densities differences not detected by routine serologic methods. The lectin microarray database can be used to correlate perturbations in ABO expression with pathologic states in addition to screening for other glycan-related disease changes. Future studies

will compare glycan expression on red blood cells in disease states and organ transplantation outcomes with what appear to be serologically compatible organs.

Statistically Significant Lectins with Comparison of ABO Blood Type						
	Pairwise Comparison					
Lectin	O vs AB	A vs AB	O vs B	O vs A	B vs AB	A vs B
EEL	X	X	X	X		X
PHA(L)	X	X	X		X	
UEA-I	X	X		X	X	
DBA	X	X		X		X
BPL	X	X			X	
UDA	X	X			X	
DSA	X	X			X	
LEL	X	X			X	
RCA120	X	X			X	
SBA	X	X				
ACA	X		X	X	X	
TJA-II	X		X	X		X
AOL	X		X	X		
ACG	X		X	X		
AAL	X		X	X		
LTL	X		X	X		
Jacalin	X		X	X		
ConA	X		X	X		
PWM	X		X			
NPA	X		X			
LCA	X			X		X
TJA-I	X					
STL	X					
WGA	X					
MAH		X	X	X	X	
ABA		X				
TxLC-I		X				