

Supplementary Material

Calculation of lectin concentration in the trench

Initial lectin concentration: 2.5 mg/mL = 2.5 µg/µL

Lectin volume used: 0.1 µL

Effective amount: 0.25 µg

Dilution factor for lectin added to trench inlet: 1/20

Effective lectin conc. after dilution: 12.5 ng/µL

Volume of trench: 200X200X100 µm = 4X10⁶ µm³ = .004 µL = 4nL

Effective lectin-holding capacity of trench at saturation: 50 pg

Induction of apoptosis

Induction of apoptosis was carried out by culturing the cells with 1µg/mL anti-IgM (Southern Biotech) for 24 h. Surface IgM (sIgM) cross-linking on such B cells is used as a model system for B Cell Receptor (BCR)-mediated antigen-induced clonal deletion, a natural process which leads to the induction of apoptosis (also known as programmed cell death) (Jiang and Clark 2001, J Immunol 166: 6025-6033).

Flow Cytometry

1×10⁵ cells were collected and washed three times with cold PBS. The cells were then resuspended in 250 µL of 10 µg/mL of labelled lectin in TBS with 1mM CaCl₂ and incubated at room temperature for 10 minutes. The cells were washed with HEPES pH 7.4 and re-suspended in 100 µL of cytometry buffer from the Apoptosis Detection Kit. The cells were further labelled with annexin V and 7AAD as per kit instructions. All samples were measured on the BD FACS Aria. All analyses were performed using FlowJo software (Tree Star Inc., 92 Ashland, OR, USA).

Cytometry data was gated to remove debris and doublets. Healthy cells were defined as being annexin V / 7AAD negative, early apoptotic cells were defined as annexin V positive / 7AAD negative, late apoptotic cells and necrotic cells were defined as 7AAD positive and the percentage of lectin-positive cells in each of these subpopulations was determined.

Supplementary Tables

Lectin	Acronym	Organism	Affinity
Concanavalin A	ConA	Canavalia exnsiformis	Branched α -mannosidic structures, Core mannose, bianttenary complex type N-glycans
Narcissus Psuedonarcissus lectin	NPL	Narcissus Psuedonarcissus (daffodils)	α -linked mannose, preferring polymannose structures containing (a-1,6) linkages
Lens culinaris agglutinin	LCA	Lens culinaris (lentil)	α -linked fucosylated mannans
Wheat germ agglutinin	WGA	Wheat	N-acetyl-D-glucoamine, sialic acid
Erythrina cristagalli lectin	ECL	Erythrina cristagalli seeds	Galactosyl- β -1,4-N-acetyl-D-glucosamine

Supplementary Table 1: Lectin specificity information.

Lectin name	Total Cell Count	Cell count w/o debris / doublets	Healthy cells %age	Healthy Cells Lectin Signal	Early apoptotic cells %age	Early apoptotic cells Lectin Signal	Late apoptotic / necrotic Cells %age	Late apoptotic / necrotic Cells Lectin Signal	Necrotic Cells %age	Necrotic Cells Lectin Signal
ConA	1.00E4	9202	62.9	342	7.67	364	24	1356	5.44	1030
ECL	6272	5517	15.8	9192	7.11	6541	59.9	4878	17.3	5380
GSL II	1.00E4	7948	63.3	172	5.46	157	22.1	159	9.16	699
LCA	6192	5078	19.2	6850	8.51	6411	52.5	5523	19.8	7765
NPL	1.00E4	9159	66.2	317	5.5	273	21.3	978	6.95	5626
SBA	7220	6130	16.3	8625	6.61	7927	60.9	3509	16.2	2289
WGA	7863	6451	13.9	1.88E4	9.78	1.76E4	61.5	1.58E4	14.8	1.95E4

Supplementary Table 2: Flow Cytometry. Total cell count is the number of cells counted by the cytometer, where this is less than 10E4 this is due to cell damage during sample preparation. Exclusion of doublets and debris is performed by FSC-A/FSC-H gating. Necrotic cells show 7-AAD staining but not Annexin-V staining. Late apoptotic cells show both 7-AAD and Annexin-V staining. Early apoptotic cells show Annexin-V staining only. Healthy cells have neither Annexin-V nor 7-AAD staining. The lectin signal is the median signal across the quadrant of the V-450 labelled lectin.