Supplemental information:

β-glucans are Masked but Contribute to Pulmonary Inflammation During *Pneumocystis* Pneumonia

Geetha Kutty[1], A. Sally Davis[2], Gabriela A. Ferreyra[1], Ju Qiu[3], Da Wei Huang[3], Monica Sassi[1], Lisa Bishop[1], Grace Handley[1], Brad Sherman[3], Richard Lempicki[3] , and Joseph A. Kovacs[1]

[1]Critical Care Medicine Department, NIH Clinical Center, National Institutes of Health, Bethesda, Maryland. [2]Diagnostic Medicine/Pathobiology, Kansas State University College of Veterinary Medicine, Manhattan, KS 66506, USA. [3]Laboratory of Immunopathogenesis and Bioinformatics, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702, USA

Supplemental Methods

Microarray hybridization and analysis. Total RNA was isolated from lung tissues using RNeasy Mini kit (QIAGEN) following the manufacturer's instructions. RNA quality was evaluated using RNA 6000 Nano LabChip (Agilent 2100 Bioanalyzer, Santa Clara, CA). Double stranded cDNA was synthesized from 150 ng of total RNA, which was then transcribed in vitro to make cRNA followed by sense strand cDNA synthesis using WT Expression Kit (Ambion). cDNA was fragmented, labeled using Affymetrix GeneChip WT Terminal Labeling Kit. Labeled cDNA was hybridized for 18 hours to the GeneChip Mouse Gene 1.0 ST arrays (Affymetrix, Santa Clara, CA) following the manufacturer's directions. Arrays were stained and washed in the Affymetrix Fluidics Station 400 and scanned (Affymetrix 7G).

Immunohistochemical staining. *P. murina*-infected and uninfected lung tissue sections were labeled for macrophages with rat anti-mouse F4/80 (Clone:A3-1) monoclonal antibody (AbD Serotec; Raleigh, NC) using a modified avidin-biotin complex immunohistochemistry technique. Briefly, 5 μm thick, HistoChoice fixed, paraffin-embedded tissue sections were deparaffinized, rehydrated, avidin and biotin blocked with the Avidin/Biotin Blocking Kit (Vector Labs; Burlingame, California), and protein blocked with X0909 (Dako; Carpinteria, CA); the primary antibody was applied at 1:50 dilution in diluent S0809 (Dako), detected with Biotin-SP AffiniPure Rabbit Anti-Rat IgG (Jackson Immunoresearch; West Grove, PA), and visualized with VECTASTAIN ABC Kit (Rabbit IgG) and 3,3'-diaminobenzidine (Vector Labs) counterstained with Mayer's hematoxylin. Three lungs from mice with *P. murina* infection that were treated with

caspofungin, 10 mg/kg, for 21 days, three lungs from untreated mice with *P. murina* infection (all from experiment 6) and two lungs from uninfected control mice were labeled with F4/80 in one run. For each lung, the number of F4/80-labeled macrophages in ten random high power fields (400X), excluding areas with large airways, were manually counted and averaged.

Table S1. Summary of experiments with caspofungin treatment of mice with *Pneumocystis* pneumonia.

Experiment and Group	Caspofungin dose	N	Organism load, log_{10}/mg tissue
Experiment 1			
Control	None	5	5.07
Caspofungin	30 µg/kg x 3 days	5	5.04
Experiment 2			
Control	None	5	5.48
Caspofungin	30 µg/kg x 3 days	5	5.62
Experiment 3			
Control	None	3	4.85
Caspofungin	30 µg/kg x 9 days	4	4.73
Experiment 4			
Control	None	5	5.24
Caspofungin	30 µg/kg x 3 days	5	5.22
Experiment 5			
Control	None	5	5.06
Caspofungin*	10 mg/kg x 9 days	4	4.80
Experiment 6			
Control	None	5	5.19
Caspofungin*	10 mg/kg x 21 days	6	3.68 †

* Caspofungin was administered 5 days per week

† $p < 0.001$

Table S2. Probe sets that were differentially expressed in caspofungin-treated mice compared to untreated *Pneumocystis*-infected mice in experiment 3.

Transcript Cluster ID	Gene Symbol	Fold change, log_2
10576235	Dpep1	1.38
10344566		1.30
10427436	C7	1.30
10549647	Ncr1	1.14
10338533		1.02
10340905		0.98
10394534	Osr1	0.93
10366586	Ifng	0.91
10565994	Art2b	0.91
10368240	Tcf21	0.90
10421186	Gm10002	0.89
10343455		0.88
10552406	Nkg7	0.85
10341146		0.85
10412211	Gzma	0.85
10395409	Meox2	0.85
10390328	Tbx21	0.84
10343852		0.79
10433776	Snai2	0.79
10344539		0.79
10342423		0.78
10343362		0.76
10340949		0.76
10338694		0.76
10344551		0.74
10343378		0.73
10342663		0.72
10417526	Dnase1l3	0.72
10338959		0.72
10414470	Tlr11	0.71
10414548	Rnase6	0.70
10338660		0.70
10567863	Cd19	0.70
10338134		0.69
10390691	Nr1d1	0.69
10343684		0.68
10509577	Pla2g2d	0.68
10342284		0.67
10475845	Acoxl	0.67
10584628	Thy1	0.67
10341150		0.66
10341684		0.66
10437655	Nubp1	0.66
10338826		0.66
10341955		0.66
10342534		0.65
10468898	Lax1	0.64

Transcript Cluster ID	Gene Symbol	Fold change, log_2
10343311		0.64
10343490		0.64
10465059	Ctsw	0.64
10343187		0.63
10342431		0.63
10478884	Snai1	0.62
10342565		0.62
10340126		0.62
10343628		0.61
10342656		0.61
10549635	Lilra5	0.61
10598507	Slc38a5	0.61
10394054	Cd7	0.60
10339999		0.60
10344401		0.60
10455901	Slc27a6	0.60
10439744	Cd96	0.59
10341513		0.59
10415017		0.59
10388086	Nlrp1c-ps	0.59
10339256		0.58
10338889		0.58
10339303		0.58
10542140	Klrb1f	0.57
10340591		0.57
10490491	Gata5	0.57
10339213		0.57
10338115		0.57
10342848		0.57
10340274		0.57
10344564		0.57
10342236		0.56
10542181	Clec9a	0.56
10579894	Hhip	0.55
10404404	Foxf2	0.55
10604057	Sept6	0.55
10547894	Cd4	0.55
10339271		0.55
10568202	Sept1	0.55
10340186		0.55
10597273	Rtp3	0.55
10343185		0.55
10338966		0.55
10472235	Dapl1	0.54
10409567	Tifab	0.54
10496438	Adh1	0.54
10382228	Axin2	0.54
10371379	Nuak1	0.54
10406928	Cd180	0.53
10402560	A130014H13Rik	0.53
10593024	Cd3e	0.53

Transcript Cluster ID	Gene Symbol	Fold change, log_2
10343717		0.52
10500529	Phgdh	0.52
10368644	Fam26f	0.51
10341632		0.51
10339232		0.51
10339767		0.51
10361215	Traf3ip3	0.51
10341573		0.51
10343937		0.50
10343082		0.50
10563355	Sec1	-0.50
10344439		-0.50
10341278		-0.50
10344032		-0.50
10339742		-0.51
10493548	Pmvk	-0.51
10344446		-0.51
10588577	Cish	-0.51
10467206	Ppp1r3c	-0.51
10367440	Itga7	-0.52
10534667	Serpine1	-0.52
10519497	Steap4	-0.52
10416230	Tnfrsf10b	-0.52
10523048	Npffr2	-0.52
10472136	Galnt13	-0.52
10344216		-0.52
10607712	Grpr	-0.52
10362138	Vnn1	-0.53
10342877		-0.53
10439321	Slc15a2	-0.53
10342437		-0.53
10542066	Tspan11	-0.53
10433885	Cebpd	-0.53
10369290	Ddit4	-0.53
10400304	Egln3	-0.53
10341075		-0.53
10531887	Slc10a6	-0.54
10414192	Mat1a	-0.54
10445875	Btg3	-0.54
10343215		-0.54
10340855		-0.54
10503334	Gem	-0.54
10343550		-0.54
10458382	Cd14	-0.55
10454198	Rnf125	-0.55
10403312	Akr1c19	-0.55
10448117	Has1	-0.55
10339543		-0.55
10342172		-0.55
10440419	Btg3	-0.55
10376513	Nlrp3	-0.55

Transcript Cluster ID	Gene Symbol	Fold change, log_2
10586614	C2cd4b	-0.56
10583044	Mmp13	-0.56
10437151	Kcnj15	-0.56
10457585		-0.56
10510580	Tnfrsf9	-0.56
10523175	Ereg	-0.56
10404840	Cd83	-0.56
10463737	Ina	-0.56
10456296	Malt1	-0.56
10339960		-0.57
10340381		-0.57
10431051	Scube1	-0.58
10575074	Tmco7	-0.58
10343662		-0.58
10338406		-0.58
10341011		-0.59
10339407		-0.59
10339552		-0.59
10420891	Scara3	-0.59
10574213	Ccl22	-0.59
10548735	Dusp16	-0.60
10504838	Nr4a3	-0.60
10476740	Slc24a3	-0.60
10344080		-0.60
10351455	Rgs5	-0.60
10342452		-0.61
10554819	Me3	-0.61
10339806		-0.61
10521038	Slc5a1	-0.61
10435704	Cd80	-0.61
10362426	Trdn	-0.61
10523134	Pf4	-0.62
10375313	Ccnjl	-0.62
10420114	Tgm1	-0.62
10597758	Csrnp1	-0.63
10576757	Fcer2a	-0.63
10459602	Ptpn2	-0.63
10338480		-0.63
10339042		-0.64
10608637		-0.64
10425283	Maff	-0.65
10462442	Il33	-0.65
10338793		-0.66
10339482		-0.66
10440522	Adamts1	-0.66
10357155	Inhbb	-0.66
10341523		-0.66
10575095	Has3	-0.67
10487597	Il1b	-0.67
10344523		-0.67
10505445	Orm3	-0.67

Transcript Cluster ID	Gene Symbol	Fold change, log_2
10523151	Cxcl1	-0.68
10449741	Sik1	-0.68
10422227	Spry2	-0.68
10342529		-0.69
10343657		-0.69
10426368	Lrrk2	-0.69
10351463	Rgs5	-0.69
10436100	Retnlg	-0.70
10520862	Fosl2	-0.70
10362129	Vnn3	-0.71
10343306		-0.71
10343497		-0.73
10340424		-0.75
10500710	BC037703	-0.76
10402347	Ifi27l2a	-0.77
10386455	Rasd1	-0.79
10492448	Ptx3	-0.79
10340645		-0.79
10576386	Rhou	-0.80
10487588	Il1a	-0.80
10399854	Slc26a4	-0.80
10547206	Fxyd4	-0.80
10409278	Nfil3	-0.81
10548118	Prmt8	-0.81
10435712	Cd80	-0.82
10536563	Cftr	-0.84
10382321	Kcnj2	-0.84
10513722	Tnfsf15	-0.84
10377439	Per1	-0.86
10341833		-0.86
10523672		-0.88
10520452	Il6	-0.91
10379721	Ccl4	-0.94
10542319	Apold1	-0.97
10373912	Osm	-0.98
10482772	Nr4a2	-1.00
10552743	Il4i1	-1.02
10408529		-1.03
10545212	Gm5574	-1.06
10523182	Areg	-1.08
10462132	E030010A14Rik	-1.09
10545202	Gm1077	-1.11
10445746	Trem1	-1.13
10574226	Ccl17	-1.32
10459455	Alpk2	-1.34
10523156	Cxcl2	-1.40
10419568	Ear11	-1.43
10545247	Igk-V19-14	-1.91