

Supplementary Methods

Phagocytosis

Opsonize *E. coli* with serum (for complement), or with heat inactivated serum (for IgG).

1. 500 μ L FITC-labelled *E. coli* (1×10^9 /ml) + 100 μ L serum
2. Incubate at 37C for 30 min
3. Centrifuge for 8 min at 2800 g
4. Remove supernatant and wash with PBS, then centrifuge for 8 min at 2800 g
5. Resuspend pellet in 0.5 mL PBS

Phagocytosis assay

1. 300 μ L heparin blood (24×10^5 cells) + 24×10^5 *E. coli* (in 240 μ L) and mix well
2. Incubate samples for 5, 15 and 30 min at 37C. Incubate negative controls at 4C (on ice).
3. Store samples on ice after incubation.
4. Add 100 μ L cold trypan blue to each tube and mix
5. Wash cells twice with PBEA, centrifuge for 8 min at 2800 g
6. Remove supernatant, add 1ml 1 x ELB solution and incubate on ice for 30min, mix every 5 min
7. Centrifuge tubes for 8 min at 2800 g
8. Wash pellet with PBEA
9. Resuspend cells with 300 μ L FACS flow buffer and measure with flow cytometer.

Supplementary data

Figure S1. WURSS21 question “how sick do you feel today?”

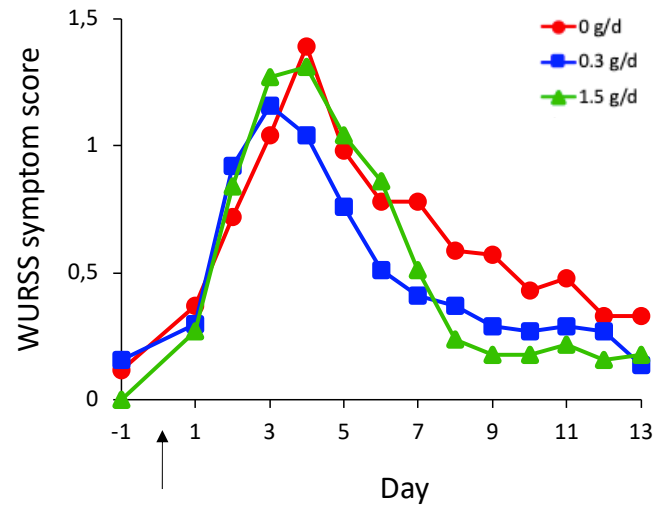


Figure S1. Time course of WURSS-21 question 1 “how sick do you feel today” ($p < 0.0001$).

Supplementary data

Table S1. Phagocytic capacity was evaluated in two distinct ways: 1) per interval of incubation (change between d-55 and d-1; incubation for 5, 15 and 30 minutes) and 2) per complete interval of incubation (change between d-55 and d-1; incubation 5 to 30 minutes), and measured as % of cells phagocytosing fluorescently labeled *Ecoli* and number of labeled bacteria in phagocytes (mean fluorescence intensity, MFI), in three opsonization conditions (IgG, IgG + complement, none).

Granulocytes % phagocytosis

		Non -opsonized						IgG-opsonized						IgG+ complement-opsonized					
		5 min		15 min		30 min		5 min		15 min		30 min		5 min		15 min		30 min	
Day	N	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0 g/day																			
-55	60	1,3%	0,3%	11,3%	0,9%	32,8%	1,3%	1,8%	0,3%	20,0%	1,3%	46,6%	1,5%	1,5%	0,3%	17,6%	1,1%	44,4%	1,5%
-1	60	1,9%	0,3%	12,1%	0,8%	34,3%	1,5%	2,4%	0,3%	20,5%	0,9%	47,3%	1,4%	2,4%	0,3%	18,5%	1,0%	44,0%	1,5%
0.3 g/day																			
-55	58	2,0%	0,3%	13,0%	1,2%	36,0%	1,6%	2,5%	0,4%	23,7%	1,3%	50,0%	1,7%	2,1%	0,3%	19,4%	1,1%	44,5%	1,5%
-1	58	2,1%	0,3%	13,1%	0,9%	34,6%	1,5%	2,3%	0,3%	20,6%	1,0%	47,1%	1,3%	2,1%	0,3%	18,4%	0,9%	43,0%	1,6%
1.5 g/day																			
-55	59	1,8%	0,3%	12,9%	1,0%	38,6%	1,6%	2,9%	0,4%	22,2%	1,4%	50,0%	1,7%	1,9%	0,3%	18,5%	1,2%	45,3%	1,7%
-1	59	1,3%	0,2%	11,8%	0,9%	35,8%	1,6%	2,0%	0,3%	20,6%	1,0%	49,5%	1,5%	1,6%	0,3%	18,0%	1,0%	44,7%	1,7%

Monocytes % phagocytosis

		Non -opsonized						IgG-opsonized						IgG+ complement-opsonized					
		5 min		15 min		30 min		5 min		15 min		30 min		5 min		15 min		30 min	
Day	N	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0 g/day																			
-55	60	1,7%	0,3%	7,0%	0,6%	14,2%	0,9%	1,5%	0,2%	9,8%	0,7%	19,9%	0,9%	1,5%	0,3%	9,4%	0,6%	18,7%	0,8%
-1	60	2,0%	0,3%	7,5%	0,6%	15,0%	0,8%	1,7%	0,3%	8,7%	0,7%	18,8%	1,1%	2,1%	0,3%	9,0%	0,6%	19,0%	0,9%
0.3 g/day																			
-55	58	2,2%	0,4%	8,5%	0,8%	17,7%	1,0%	2,2%	0,3%	12,1%	0,7%	21,8%	1,2%	2,1%	0,3%	10,0%	0,7%	19,3%	1,1%
-1	58	3,0%	0,4%	8,5%	0,7%	16,7%	0,9%	2,2%	0,3%	10,6%	0,6%	21,1%	0,9%	2,8%	0,5%	10,4%	0,8%	20,7%	1,1%
1.5 g/day																			
-55	59	2,3%	0,5%	8,7%	0,8%	17,8%	1,2%	2,4%	0,4%	11,4%	1,0%	22,3%	1,3%	2,0%	0,5%	9,8%	0,8%	21,0%	1,1%
-1	59	2,0%	0,3%	7,1%	0,6%	15,5%	0,9%	1,4%	0,2%	9,2%	0,5%	22,2%	1,0%	1,7%	0,3%	8,4%	0,6%	19,6%	1,0%

Granulocytes MFI

		Non -opsonized						IgG-opsonized						IgG+ complement-opsonized					
		5 min		15 min		30 min		5 min		15 min		30 min		5 min		15 min		30 min	
Day	N	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0 g/day																			
-55	60	34	8	403	40	1559	105	52	10	783	73	2341	152	43	11	689	60	2197	143
-1	60	38	6	411	29	1550	82	62	8	780	48	2271	122	56	7	662	41	2025	106
0.3 g/day																			
-55	58	51	10	479	55	1737	142	73	13	970	82	2628	178	59	10	756	64	2200	148
-1	58	45	7	428	31	1554	82	57	8	795	60	2227	124	52	8	671	48	1945	109
1.5 g/day																			
-55	59	36	7	445	41	1813	116	71	11	874	72	2533	172	50	10	696	59	2218	145
-1	59	29	6	384	29	1559	89	51	9	757	49	2294	111	43	9	660	47	2006	118

Monocytes MFI

		Non -opsonized						IgG-opsonized						IgG+ complement-opsonized					
		5 min		15 min		30 min		5 min		15 min		30 min		5 min		15 min		30 min	
Day	N	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0 g/day																			
-55	60	44	7	193	20	494	34	45	7	277	25	696	49	42	9	264	21	494	34
-1	60	43	6	199	18	505	28	41	6	226	18	629	43	53	7	226	19	505	28
0.3 g/day																			
-55	58	52	13	239	26	663	53	59	8	359	30	838	65	64	13	275	26	663	53
-1	58	69	10	203	18	546	35	53	8	286	20	704	42	64	11	255	24	546	35
1.5 g/day																			
-55	59	60	16	267	32	681	70	75	15	323	33	841	77	67	18	285	27	681	70
-1	59	47	7	186	14	475	24	37	6	228	15	719	45	39	6	230	17	475	24

The outcome showed that the longer the interval of incubation the higher the percentage of cells (either granulocytes or monocytes) having phagocytized *Ecoli* and the higher the MFI ($p < 0.001$); the batch of *Ecoli* used in the analyses demonstrated significant impacts on the outcome in percentage of cells (only granulocytes ($p < 0.03$) but not monocytes), but especially on MFI (both granulocytes and monocytes) ($p < 0.001$); the percentage of monocytes having phagocytized *Ecoli* was higher on d-55 than d-1 in the cRG-I groups ($p < 0.04$); the MFI was higher on d-55 than d-1, for both granulocytes and especially monocytes in the cRG-I groups ($p < 0.002$). Overall, cRG-I slightly but significantly (due to high power in the data) reduced the phagocytic capacity of granulocytes and monocytes.