viruses

MDPI

Supplementary Materials

Probenecid Inhibits Respiratory Syncytial Virus (RSV) Replication

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Table S1. OAT3 Expression.

HEp-2 Cells	**Probenecid Treated**	**DMSO only**
	Raw Ct Value	**Raw Ct Value**
ACTB	16.3 (95% CI 14.8-17.8)	15.3 (95% CI 14.4-16.2)
OAT3	No Ct	32.1 (95% CI 31.2-33)
BALB/c Mice	**Probenecid Treated (200 mg/kg)**	
	Fold change reduction	
OAT3	15.6 (95% CI 3.8-27.4)	
Paired Ct Values	**Probenecid Treated**	**PBS**
	Raw Ct Value	**Raw Ct Value**
ACTB	11.94	11.10
OAT3	33.40	31.26
ACTB	11.66	11.65
OAT3	33.65	30.38
ACTB	11.91	11.70
OAT3	36.06	30.72
ACTB	14.5	13
OAT3	37	31

HEp-2 cells were treated with IC_{90} concentration of probenecid [7.2uM] or mock treated (DMSO only) for 24h. OAT3 transcripts were determined as described in Methods. Since probenecid treatment resulted in undetectable levels of OAT3 transcripts, fold-change was not performed. Lung RNA was extracted at day 2 pi in mice treated with 200 mg/kg probenecid or PBS (i.e., 24h post treatment). OAT3 gene expression in 200 mg/kg probenecid treated mice was normalized to a housekeeping gene, ACTB (the gene for beta (β)-actin) which is an endogenous control and compared to PBS treated mice. The data is represented as the reciprocal of $2^{\Delta\Delta Ct}$ (fold-change decrease). Mean paired Ct values are included for ACTB and OAT3. Data represent the mean Ct values or fold-change of three individual experiments and 95% confidence intervals (CI).

Table S2. BAL cells.

A. BAL cells/mL.

Sex	**Group**	**BAL cells/mL + (SD)**					
		Day 3		**Day 5**		**Day 7**	
Males	Prophylactic 2mg/kg	1.25E+05	*3.23E+04*	1.19E+05	*3.06E+04*	1.31E+04	*3.75E+03*
	Prophylactic 200mg/kg	2.44E+05	*9.91E+04*	2.94E+05	*5.00E+04*	1.31E+04	*5.15E+03*
	Treatment 2mg/kg	1.94E+05	*8.91E+04*	1.20E+05	*5.18E+04*	1.75E+04	*9.84E+03*
	Treatment 200mg/kg	2.65E+05	*8.48E+04*	1.45E+05	*5.18E+04*	1.94E+04	*8.51E+03*
	PBS	2.44E+05	*2.39E+04*	1.00E+05	*3.06E+04*	1.95E+04	*7.98E+03*
Females	Prophylactic 2mg/kg	2.69E+05	*1.32E+05*	2.56E+05	*5.76E+04*	7.50E+04	*1.22E+05*
	Prophylactic 200mg/kg	3.08E+05	*1.26E+05*	2.80E+05	*6.75E+04*	1.25E+05	*7.71E+04*
	Treatment 2mg/kg	3.60E+05	*1.40E+05*	2.70E+05	*6.94E+04*	1.35E+05	*8.91E+04*
	Treatment 200mg/kg	3.13E+05	*1.39E+05*	1.40E+05	*1.16E+05*	9.17E+04	*3.79E+04*
	PBS	2.35E+05	*1.01E+05*	1.70E+05	*1.02E+05*	2.50E+05	*1.05E+05*

B. BAL cells types.

Sex	DPI	Cell Type	Total BAL cells (*SD*)									
			Prophylactic 2mg/kg		Prophylactic 200mg/kg		Treatment 2mg/kg		Treatment 200mg/kg		PBS	
Males	**3**	Macrophages	3.06E+04	*7.12E+03*	7.05E+04	*5.65E+04*	3.79E+04	*1.67E+04*	4.22E+04	*1.45E+04*	4.87E+04	*5.36E+03*
		Lymphocytes	5.67E+02	*4.93E+02*	1.38E+03	*1.04E+03*	7.88E+02	*5.17E+02*	9.63E+02	*4.85E+02*	4.67E+02	*4.51E+02*
		Neutrophils	4.00E+02	*1.00E+02*	1.08E+03	*1.37E+03*	0.00E+00	*0.00E+00*	6.25E+02	*2.63E+02*	1.83E+02	*3.18E+02*
		Eosinophils	1.33E+02	*2.31E+02*	8.00E+01	*1.79E+02*	1.38E+02	*1.89E+02*	0.00E+00	*0.00E+00*	6.50E+02	*2.18E+02*
	5	Macrophages	2.15E+04	*1.16E+04*	5.66E+04	*3.39E+04*	2.66E+04	*1.30E+04*	2.92E+04	*1.16E+04*	1.92E+04	*6.21E+03*
		Lymphocytes	3.90E+02	*3.75E+02*	1.25E+03	*7.58E+02*	3.63E+02	*1.25E+02*	2.38E+02	*2.10E+02*	4.60E+02	*1.95E+02*
		Neutrophils	0.00E+00	*0.00E+00*	4.63E+02	*8.28E+02*	4.25E+02	*2.50E+02*	4.63E+02	*3.13E+02*	2.10E+02	*1.52E+02*
		Eosinophils	8.00E+01	*1.00E+02*	4.38E+02	*5.06E+02*	1.13E+02	*1.65E+02*	1.50E+02	*1.60E+02*	1.00E+02	*9.35E+01*
	7	Macrophages	1.66E+04	*1.13E+04*	1.27E+04	*5.10E+03*	1.66E+04	*1.08E+04*	1.75E+04	*7.41E+03*	1.86E+04	*7.92E+03*
		Lymphocytes	6.75E+02	*5.44E+02*	2.06E+02	*1.18E+02*	4.50E+02	*4.22E+02*	1.45E+03	*1.83E+03*	6.30E+02	*3.93E+02*

		Neutrophils	3.00E+01	*7.50E+01*	4.38E+01	*8.75E+01*	8.13E+01	*1.63E+02*	6.88E+01	*1.38E+02*	2.00E+01	*4.47E+01*
		Eosinophils	0.00E+00	*0.00E+00*	0.00E+00	*0.00E+00*	0.00E+00	*0.00E+00*	0.00E+00	*0.00E+00*	0.00E+00	*0.00E+00*
Females	3	Macrophages	6.89E+04	*4.04E+04*	7.71E+04	*2.67E+04*	4.40E+04	*2.24E+04*	5.71E+04	*3.56E+04*	5.11E+04	*2.66E+04*
		Lymphocytes	2.38E+03	*1.77E+03*	2.10E+03	*1.27E+03*	4.88E+02	*5.39E+02*	1.20E+03	*7.55E+02*	1.35E+03	*1.03E+03*
		Neutrophils	1.30E+02	*0.00E+00*	3.00E+02	*4.24E+02*	4.88E+02	*8.78E+02*	1.13E+03	*1.30E+03*	6.00E+02	*8.22E+02*
		Eosinophils	5.40E+02	*5.34E+02*	5.00E+02	*7.07E+02*	0.00E+00	*0.00E+00*	6.00E+02	*3.77E+02*	0.00E+00	*0.00E+00*
	5	Macrophages	5.22E+04	*2.11E+04*	5.49E+04	*3.23E+04*	3.45E+04	*2.11E+04*	3.05E+04	*8.33E+03*	5.18E+04	*1.96E+04*
		Lymphocytes	1.45E+03	*7.68E+02*	8.50E+02	*3.66E+02*	1.20E+03	*1.04E+03*	4.63E+02	*3.04E+02*	1.80E+03	*1.07E+03*
		Neutrophils	0.00E+00	*0.00E+00*	9.00E+01	*2.01E+02*	3.75E+02	*6.24E+02*	7.50E+01	*1.50E+02*	2.80E+02	*3.90E+02*
		Eosinophils	3.30E+02	*4.50E+02*	1.70E+02	*3.80E+02*	1.63E+02	*3.25E+02*	2.50E+02	*3.79E+02*	1.60E+02	*3.58E+02*
	7	Macrophages	2.64E+04	*1.38E+04*	2.42E+04	*2.44E+04*	5.37E+04	*1.99E+04*	1.24E+04	*1.03E+04*	1.36E+04	*4.05E+03*

	Lymphocytes	4.00E+02	*3.11E+02*	2.60E+02	*2.88E+02*	6.88E+02	*3.42E+02*	1.50E+02	*1.73E+02*	2.50E+02	*3.24E+02*
	Neutrophils	1.00E+02	*2.50E+02*	3.70E+02	*5.81E+02*	1.38E+02	*2.75E+02*	0.00E+00	*0.00E+00*	1.30E+02	*1.30E+02*
	Eosinophils	1.20E+02	*2.50E+02*	2.10E+02	*2.79E+02*	4.75E+02	*5.62E+02*	0.00E+00	*0.00E+00*	3.00E+01	*6.71E+01*

BAL cells were collected from male and female BALB/c mice (n>3/group) at days 3, 5, or 7 post-infection. (A) Cells were enumerated by hemocytometer and Trypan blue and presented as BAL cells/mL (+SD; italics). (B) A portion of BAL cells were cytospun onto glass slides, fixed with 4% paraformaldehyde, and stained with hematoxylin and eosin. At least 100 cells per slide were phenotyped based on morphology, and values represent total number of each cell type in the BAL (+SD, in italics). There were no significant differences between treatment groups compared to PBS control as determined by two-way ANOVA.