

Supplementary data to:

Original article:

IMMUNOMODULATORY COMPONENTS OF *TRICHINELLA SPIRALIS* EXCRETORY-SECRETORY PRODUCTS WITH LACTOSE-BINDING SPECIFICITY

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Raw data concerning **Figure 2d. *T. spiralis* proteins recognized by anti-galectin-1 antibodies, (d) Binding of TsGal-1-like proteins to Matrigel.** Serial dilutions of TsGal-1-like proteins in MePBS or PBS (0-20 µg/ml) were added to microtiter plate wells coated with Matrigel. Bound TsGal-1-like proteins were detected with anti-human galectin-1 Ab. The summarized results of binding assay are shown as mean absorbance obtained on 450 nm ± SD. *p < 0.05

Samples (µg/ml)	MEPBS (A 450 nm)	Mean ± SD	PBS (A 450nm)	Mean ± SD
20	0.435	0.496 ± 0.05	0.314	0.298 ± 0.01*
	0.518		0.293	
	0.534		0.287	
10	0.348	0.355 ± 0.03	0.216	0.259 ± 0.05*
	0.386		0.306	
	0.330		0.255	
5	0.342	0.315 ± 0.02	0.246	0.258 ± 0.02
	0.306		0.249	
	0.296		0.280	
0	0.298	0.238 ± 0.05	0.221	0.209 ± 0.01
	0.213		0.208	
	0.204		0.198	

Raw data concerning **Figure 3. Phenotype of dendritic cells (DCs) treated with TsGal-1-like proteins** – the expression of maturation markers, analyzed by flow cytometry, is presented as percentage (%) or Mean fluorescence intensity (MFI) for each of three independent experiments, all upon the treatment of DCs with *T. spiralis* ES L1 components (50 µg/ml), TsGal-1-like proteins (5 µg/ml) and LPS/IFN-γ (200/20 ng/ml) respectively. Non-treated cells were used as controls. The specificity of TsGal-1-like proteins interaction with cell surface glycoproteins was assessed by inhibition of binding with lactose. TsGal-1-like proteins were incubated with an equal volume of 0.2 M lactose (TsGal-1-like/Lac) before introducing in DC culture, and the control for the inhibitory effect of lactose (Lac) on binding were DCs treated with 0.1 M Lac. The summarized results of HLA-DR, CD83, CD86, CD40, CCR7 and ILT3 expression by DCs are shown as mean ± SD from three different experiments. *p < 0.05, **p < 0.01, ***p < 0.005 compared to control untreated DCs (other comparisons are indicated by lines in Figure 3).

Markers on DCs	Treatment	Expression of markers on DCs	Mean ± SD
HLA DR (Expression shown as MFI)	Control	6779	9823 ± 3185.90
		13134	
		9556	
	LPS/IFN-γ	13892	19001.3 ± 4862.43**
		23572	
		19540	
ES L1	10822	14194.7 ± 4341.44	
	19093		
	12669		
TsGal-1-like	8474	11010.3 ± 2231.15	
	11887		
	12670		
TsGal-1-like/Lac	10305	13381 ± 2666.21	
	15030		
	14808		
Lac	8407	10672 ± 2407.30	
	13200		
	10409		
CD83 (Expression shown as %)	Control	7.0	9.1 ± 1.9
		10.7	
		9.6	
	LPS/IFN-γ	23.8	34.1 ± 9.65***
		42.9	
		35.7	
ES L1	11.9	19.1 ± 7.30*	
	26.5		
	18.8		
TsGal-1-like	17.1	21.4 ± 5.08**	
	27.0		
	20.1		
TsGal-1-like/Lac	14.5	17.4 ± 2.90	
	20.3		
	17.5		
Lac	7.37	10.4 ± 3.62	
	14.4		
	9.4		
CD86 (Expression shown as MFI)	Control	589	617.7 ± 47.08
		672	
		592	
	LPS/IFN-γ	923	956.0 ± 29.31***
979			
966			
		738	

	ES L1	727 707	724.0 ± 15.72*
	TsGal-1-like	645 657 630	644.0 ± 13.53
	TsGal-1-like/Lac	654 550 570	591.3 ± 55.18
	Lac	516 543 520	526.3 ± 14.57
CD40 (Expression shown as MFI)	Control	589 562 592	617.7 ± 48.07
	LPS/IFN-γ	923 979 966	956 ± 29.31***
	ES L1	738 727 707	724 ± 15.72
	TsGal-1-like	645 657 630	644 ± 13.23*
	TsGal-1-like/Lac	654 550 570	591.3 ± 55.18
	Lac	516 543 520	526.3 ± 14.57
CCR7 (Expression shown as %)	Control	9.55 13.6 10.4	11.2 ± 2.14
	LPS/IFN-γ	8.97 16.0 10.6	11.9 ± 3.68**
	ES L1	24.1 26.5 25.5	25.4 ± 1.21***
	TsGal-1-like	24.5 27.9 26.5	26.3 ± 1.71***
	TsGal-1-like/Lac	23.2 23.0 24.8	23.7 ± 0.99
	Lac	19.7 22.5 17.1	19.8 ± 2.70
ILT3 (Expression shown as %)	Control	6.4 9.9 7.7	8.0 ± 1.77
	LPS/IFN-γ	9.15 11.8 10.2	10.4 ± 1.33
	ES L1	32.5 47.1 40.4	40.0 ± 7.31***
		34.7	

	TsGal-1-like	43.6	38.3 ± 4.67***
		36.7	
	TsGal-1-like/Lac	30.2	27.0 ± 4.41**
		22.0	
		28.9	
	Lac	22.1	18.3 ± 3.60
14.8			
18.2			

Raw data concerning **Figure 4. Cytokine expression of TsGal-1-like proteins-pulsed dendritic cells (DCs)** - the expression of IL-10, IL12p40/p70 and transforming growth factor (TGF)- β analyzed by flow cytometry is presented as percentage (%) for each of three independent experiments, all upon the treatment of DCs with LPS/IFN- γ (200/20 ng/ml), *T. spiralis* ES L1 products (50 μ g/ml) and TsGal-1-like proteins (5 μ g/ml), with or without 0.1 M lactose (Lac) as inhibitor of binding. Non-treated cells were used as controls. The summarized results are presented as mean value of percentage (%) of the expression \pm SD from three different experiments. **p < 0.01. ***p < 0.005 compared to control untreated DCs (other comparisons are indicated by lines in Figure 4).

Cytokines	Treatment	Expression of cytokines in DCs	Mean \pm SD
IL-10 (Expression shown as %)	Control	3.36	4.5 \pm 1.25
		5.85	
		4.4	
	LPS/IFN- γ	5.91	9.1 \pm 2.87
		11.4	
		10.1	
ES L1	12.84	14.5 \pm 1.68***	
	16.2		
	14.4		
TsGal-1-like	17.4	19.0 \pm 1.71***	
	20.8		
	18.8		
TsGal-1-like/Lac	15.5	15.0 \pm 0.55***	
	15.0		
	14.4		
Lac	5.6	7.1 \pm 1.86	
	9.2		
	6.6		
IL-12 p40/p70 (Expression shown as %)	Control	9.4	8.2 \pm 1.16
		7.8	
		8.1	
	LPS/IFN- γ	19.5	16.5 \pm 3.48**
		11.5	
		17.4	
ES L1	11.0	11.4 \pm 0.61	
	12.1		
	11.0		
TsGal-1-like	9.1	10.7 \pm 2.12	
	13.1		
	9.9		
TsGal-1-like/Lac	12.7	11.7 \pm 0.91	
	10.9		
	11.6		
Lac	8.1	8.7 \pm 0.92	
	9.78		
	8.3		
TGF- β (Expression shown as %)	Control	40.6	41.6 \pm 1.27
		43.0	
		41.1	
	LPS/IFN- γ	28.7	30.3 \pm 1.65**
		32.0	
		30.3	
ES L1	61.8	58.4 \pm 3.62**	
	54.6		
	58.8		
		64.2	

	TsGal-1-like	70.0 66.2	66.8 ± 2.95***
	TsGal-1-like/Lac	59.4 63.6 61.1	61.4 ± 2.11***
	Lac	50.6 52.4 50.8	51.3 ± 0.99

Raw data concerning **Figure 5. T helper polarization influenced by treated DCs** - the expression of IL-4, IL-10 and IFN- γ within the CD4⁺ T cell population, analyzed by flow cytometry, is presented as percentage (%) for each of three independent experiments, all upon the co-culture of treated DCs with magnetic-activated cell sorting-purified allogenic T cells (1×10^5 /well) for 6 days in 1:20 DC:T cell ratio. The summarized results are shown as mean % \pm SD of three experiments with different DCs donors. *p < 0.05, **p < 0.01, ***p < 0.001 compared to control untreated DCs (other comparisons are indicated by lines in Figure 5).

Cytokines	Treatment	Expression of cytokines in CD4 ⁺ T cells	Mean \pm SD
IL-4 (Expression shown as %)	Control	12.4	9.81 \pm 2.89
		6.9	
		8.1	
	LPS/IFN- γ	14.0	10.1 \pm 3.51
		7.16	
		9.2	
ES L1	24.8	21.4 \pm 3.45***	
	17.9		
	21.4		
TsGal-1-like	19.2	16.2 \pm 3.61**	
	12.2		
	17.2		
TsGal-1-like/Lac	16.2	12.6 \pm 5.58	
	6.2		
	15.5		
Lac	14.2	8.4 \pm 5.07	
	4.8		
	6.2		
IL-10 (Expression shown as %)	Control	14.9	10.1 \pm 4.19
		7.14	
		8.3	
	LPS/IFN- γ	15.4	10.6 \pm 4.27
		7.13	
		9.4	
ES L1	22.0	19.4 \pm 3.35***	
	15.6		
	20.5		
TsGal-1-like	21.4	19.2 \pm 2.31***	
	16.8		
	19.5		
TsGal-1-like/Lac	16.7	15.3 \pm 2.34*	
	12.6		
	16.6		
Lac	15.0	10.8 \pm 3.65	
	8.4		
	9.1		
IFN- γ (Expression shown as %)	Control	4.16	4.3 \pm 0.52
		4.91	
		3.9	
	LPS/IFN- γ	11.5	12.1 \pm 0.60***
12.7			
12.2			
ES L1	5.79	5.0 \pm 0.76	
	4.29		
	4.8		
TsGal-1-like	5.56	4.4 \pm 1.16	
	3.25		

		4.5	
		6.0	
	TsGal-1-like/Lac	4.77	5.3 ± 0.65
		5.0	
	Lac	5.4	4.3 ± 0.97
		3.5	
		4.1	

Raw data concerning **Figure 6. Tolerogenic properties and functions of TsGal-1-like proteins-treated DCs** - the expression of Foxp3 in CD4⁺CD25⁺ T cell population, as well as IL-10 and TGF-β within the same T cell population, analyzed by flow cytometry, is presented as percentage (%) for each of three independent experiments, all upon the co-culture of treated and non-treated DCs with magnetic-activated cell sorting-purified allogenic T cells (Tly) (1 × 10⁵/well) for 3 days in 1:50 DC:T cell ratio and then re-stimulated with interleukin (IL)-2 (2 ng/ml) for another 3 days. The summarized results for the expression of CD4⁺CD25⁺Foxp3⁺ T regulatory cells and IL-10 and TGF-β within CD4⁺CD25⁺ T cells are shown as the mean % ± SD from three different experiments. **p < 0.01, ***p < 0.005 compared to control untreated DCs (other comparisons are indicated by lines in Figure 6).

Transcriptional regulator or cytokine	Treatment	Expression of proteins in CD4 ⁺ CD25 ⁺ T cells	Mean ± SD
Foxp3 (Expression shown as %)	Control	3.24	4.2 ± 0.83
		4.5	
		4.8	
	LPS/IFN-γ	3.38	4.7 ± 1.13
		5.5	
		5.1	
ES L1	15.8	12.9 ± 2.50***	
	11.2		
	11.8		
TsGal-1-like	11.6	11.8 ± 1.11***	
	13.0		
	10.8		
TsGal-1-like/Lac	10.8	9.7 ± 1.01***	
	9.6		
	8.8		
Lac	5.9	5.4 ± 0.50	
	4.9		
	5.5		
IL-10 (Expression shown as %)	Control	11.0	9.6 ± 2.06
		7.2	
		10.5	
	LPS/IFN-γ	15.5	15.4 ± 1.40
		14.0	
		16.8	
ES L1	18.5	23.8 ± 4.73***	
	27.5		
	25.5		
TsGal-1-like	25.8	26.3 ± 2.83***	
	29.3		
	23.7		
TsGal-1-like/Lac	22.1	19.8 ± 2.08**	
	19.1		
	18.1		
Lac	3.8	12.1 ± 7.26	
	17.1		
	15.5		
TGF-β (Expression shown as %)	Control	35.9	37.3 ± 4.56
		42.4	
		33.6	
	LPS/IFN-γ	40.1	43.7 ± 4.48
		48.7	
		42.2	
		70.7	

	ES L1	73 72.1	71.9 ± 1.19***
	TsGal-1-like	80 74 70.1	74.7 ± 4.99***
	TsGal-1-like/Lac	67.8 63.6 64.4	65.3 ± 2.23**
	Lac	50.1 55.4 48.2	51.2 ± 3.73