

**Intact interleukin-10 receptor signaling protects from hippocampal damage
elicited by experimental neurotropic virus infection of SJL mice**

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Supplemental table S1

Summary of primer pairs used for RT-qPCR (brain)

	Gene	Primer sequence		length
<i>Interleukin-1α</i>	<i>Il1α</i>	F	AAG CAA CGG GAA GAT TCT GA	179 bp
		R	TGA CAA ACT TCT GCC TGA CG	
<i>Interleukin-2</i>	<i>Il2</i>	F	GCA GGA TGG AGA ATT ACA GGA	183 bp
		R	TGA AAT TCT CAG CAT CTT CCA A	
<i>Interleukin-4</i>	<i>Il4</i>	F	CCT CAC AGC AAC GAA GAA CAC C	133 bp
		R	CAT CGA AAA GCC CGA AAG AGT C	
<i>Interleukin-5</i>	<i>Il5</i>	F	ATG GAG ATT CCC ATG AGC AC	180 bp
		R	CCC ACG GAC AGT TTG ATT CT	
<i>Interleukin-6</i>	<i>Il6</i>	F	GTT CTC TGG GAA ATC GTG GA	176 bp
		R	CCA GAG GAA ATT TTC AAT AGG C	
<i>Interleukin-10</i>	<i>Il10</i>	F	CCA AGC CTT ATC GGA AAT GA	162 bp
		R	TTT TCA CAG GGG AGA AAT CG	
<i>Forkhead box P3</i>	<i>Foxp3</i>	F	TTC-TCA-CAA-CCA-GGC-CAC-TTG	88 bp
		R	CCC-AGG-AAA-GAC-AGC-AAC-CTT	
<i>Tumor necrosis factor</i>	<i>Tnf</i>	F	GCC TCT TCT CAT TCC TGC TT	203 bp
		R	CAC TTG GTG GTT TGC TAC GA	
<i>Interferon-γ</i>	<i>Ifnγ</i>	F	CAC GGC ACA GTC ATT GAA AG	144 bp
		R	AAT CTG GCT CTG CAG GAT TT	
<i>Transforming growth factor-β1</i>	<i>Tgfβ1</i>	F	TTG CTT CAG CTC CAC AGA GA	183 bp
		R	TGG TTG TAG AGG GCA AGG AC	
<i>Theiler's murine encephalomyelitis virus</i>	<i>TMEV</i>	F	GAC TAA TCA GAG GAA CGT CAG C	129 bp
		R	GTG AAG AGC GGC AAG TGA GA	
<i>glyceraldehyde-3-phosphate dehydrogenase</i>	<i>Gapdh</i>	F	GAG GCC GGT GCT GAG TAT GT	288 bp
		R	GGT GGC AGT GAT GGC ATG GA	
<i>hypoxanthine guanine phosphoribosyl transferase</i>	<i>Hprt</i>	F	GGA CCT CTC GAA GTG TTG GA	169 bp
		R	TTG CGC TCA TCT TAG GCT TT	
<i>β-Actin</i>	<i>Actβ</i>	F	GGC TAC AGC TTC ACC ACC AC	233 bp
		R	ATG CCA CAG GAT TCC ATA CC	

bp = base pairs; F = forward; R = reverse

Supplemental table S2

Summary of primer pairs used for targeted RT-qPCR screening (spleen)

Gene name	Gene symbol	mRNA NCBI reference sequence	Primer sequence	
Interleukin 4	Il4	NM_021283.2	F	ATTCATCGATAAGCTGCAC
			R	GTA CTACGAGTAATCCATTTGC
Interleukin 10	Il10	NM_010548.2	F	TGATTTTAATAAGCTCCAAGAC
			R	TGTTTTAGCTTTTCATTTTGAT
Tumor necrosis factor	Tnf	NM_013693.3	F	TCAGATCATCTTCTCAA AATTC
			R	ACTAGTTGGTTGTCTTTGAGAT
Interferon β 1	Ifn β 1	NM_010510.1	F	GACAGTACTAGAGGAAAAGCAA
			R	AGCTGTTGTACTTCATGAGTTT
Interferon γ	Ifn γ	NM_008337.3	F	CTATTTTAACTCAAGTGGCATAG
			R	TTCAAAGAGTCTGAGGTAGAAA
Interferon alpha and beta receptor subunit 2	Ifnar2	XM_006522923.2	F	ATGAACCTTGCACTATAAACAT
			R	GTCTTTGCTCATGACTGTGTA
Stimulator of interferon genes	Sting	NM_028261.1	F	GTCTAGGAAGCAGAAGATGC
			R	CAGAAAGATGAGGGCTACATA
Cyclic GMP-AMP synthase	cGas	NM_173386.5	F	GTATTATCAGCTACCAAGATGC
			R	TCCACACTGACATCTATATCTTT
TANK binding kinase	Tbk1	NM_019786.4	F	GAACTGGTTAAGGATGATTACA
			R	CAACTTCTCATACTTTTCACTA
Interferon regulatory factor 3	Irf3	NM_016849.4	F	TAACTCTGTGGTTCTGCAT
			R	ACATGTAGGAACAACCTTGAC
Tripartite motif containing 21	Trim21	NM_001082552.2	F	GAGGACCCTGGTTAGATTC
			R	ATACTCATAGGCTCCACCAT
Interferon gamma inducible protein 16	Ifi16	NM_008329.2	F	GAGATCAGAAGTAACAGGAGAA
			R	GCTTGTAGTTGATGTAGGTGTT
C-C motif chemokine ligand 2	Ccl2	NM_011333.3	F	AAGAAGCTGTAGTTTTTGTAC
			R	AGGTTTTTAATGTATGTCTGGA
C-C motif chemokine ligand 3	Ccl3	NM_011337.2	F	GTGTCATTTTCCTGACTAAGAG
			R	AGTTCCAGGTCAGTGATGTAT
C-C motif chemokine ligand 5	Ccl5	NM_013653.3	F	CAATCTTGCAAGTCGTGTTT
			R	TATCCTAGCTCATCTCCAAATA
Stromal cell-derived factor 1 alpha	Sdf1 α	NM_013655.4	F	GTTCTTCCATTTGTGTACTCTG
			R	GACTCCTGGTTTAGCTGATAG

Supplemental table S2: continued

Gene name	Gene symbol	mRNA NCBI reference sequence	Primer sequence	
<i>C-C chemokine receptor 1</i>	<i>Ccr1</i>	NM_009912.4	F	CTTGAACCTTGAATTCATAAAG
			R	AAAGACAGTGAGTCTGTGTTTC
<i>C-C chemokine receptor 2</i>	<i>Ccr2</i>	NM_009915.2	F	ATAAAGGAGCCATACCTGTAAA
			R	CATATTATTGTCTTCCATTTCC
<i>C-C chemokine receptor 3</i>	<i>Ccr3</i>	NM_009914.4	F	ATGATGTTTACTACCTGACTGG
			R	TCTACTTGTCTCTGGTGAATTT
<i>C-X-C motif chemokine receptor 4</i>	<i>Cxcr4</i>	NM_009911.3	F	AGGTAGCAGTGAAACCTCTG
			R	AAGTGTATATACTCACACTGATCG
<i>Chemokine (C-X3-C motif) receptor 1</i>	<i>Cx3cr1</i>	NM_009987.4	F	ACGGTGTCAACCATTAGTCT
			R	CTTTCTCTTTGTGAACATGAAC
<i>CD68 molecule</i>	<i>Cd68</i>	NM_001291058.1	F	GATTCAAACAGGACCTACATC
			R	CTCTCGAAGAGATGAATTCTG
<i>CD40 molecule</i>	<i>Cd40</i>	NM_011611.2	F	ATGTCATCTGTGGTTTAAAGTC
			R	CTTGACCACCTTTTTGATATAG
<i>CD206 molecule</i>	<i>Cd206</i>	NM_008625.2	F	TGGTTATCCAAATAACTTCATC
			R	CAAAAATTTTAAAGCACTTGTT
<i>Repulsive guidance molecule family member a</i>	<i>Rgma</i>	NM_177740.5	F	CTCTCATCGACAATAATTACCT
			R	GAAGATGATGGTGAGCTTG
<i>Leucine-rich repeats and immunoglobulin-like domains 1</i>	<i>Lrig1</i>	NM_008377.2	F	ATCACTGATGTGAAAATAGACG
			R	AGGATGGAGTTTCTAAGACTGT
<i>Arginase1</i>	<i>Arg1</i>	NM_007482.3	F	AGCCAAAGTCCTTAGAGATTAT
			R	CTCACGTCATACTCTGTTTCTT
<i>Resistin like alpha</i>	<i>Fizz1</i>	NM_020509.3	F	GATGAAGACTACAACCTTGTTCC
			R	AGGGATAGTTAGCTGGATTG
<i>Chitinase 3-like 3</i>	<i>Chi3l3</i>	NM_009892.2	F	GAATCTGTGGAGAAAGACATT
			R	TAGTCAAGAGACTGAGACAGTT
<i>Toll-like receptor 2</i>	<i>Tlr2</i>	NM_011905.3	F	CAGGATCTTGTTTCTGAGTGT
			R	CTGAGATTTGACGCTTTGT
<i>Toll-like receptor 3</i>	<i>Tlr3</i>	NM_126166.4	F	AGAAGATAAAGCGAGTTTCAC
			R	AAGCCAAGTACATTCTGATTT
<i>Toll-like receptor 9</i>	<i>Tlr9</i>	NM_031178.2	F	CTCTCCATACACTGAACTCTTC
			R	CATAGAGAAAGTTCTCGCTTAG

F = forward; R = reverse

Supplemental table S3

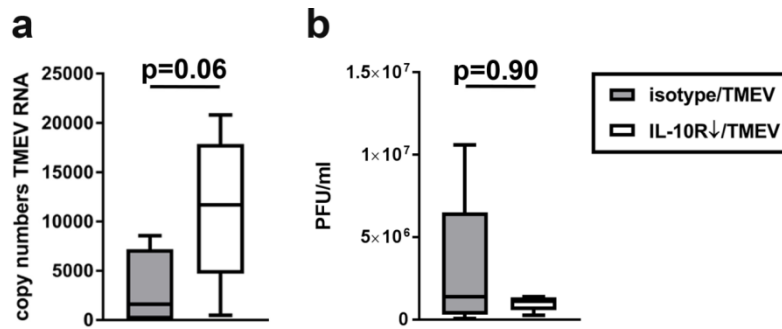
Summary of statistical analyzes: IL-10R blockade during Theiler's murine encephalomyelitis virus (TMEV) infection at 7 days post infection; p-values			
Investigation	TMEV-infected SJL mice with and without IL-10R blockade	TMEV-infected C57BL/6 and SJL mice	TMEV-infected C57BL/6 mice and SJL mice with IL-10R blockade
Histology (H&E)			
Hypercellularity (semiquantitative)	↑ 0.016*	↑ 0.016*	0.056
Immunohistochemistry, hippocampus			
CD3	↑ 0.032*	↑ 0.016*	↑ 0.016*
CD45R	↑ 0.016*	↑ 0.032*	0.222
Foxp3	↑ 0.032*	0.413	↓ 0.016*
CD107b	0.1111	↑ 0.016*	↑ 0.032*
Arginase-1	↑ 0.032*	↑ 0.032*	0.151
β-APP	↑ 0.016*	↑ 0.016*	0.056
NeuN	↓ 0.032*	↓ 0.016*	0.222
TMEV	0.921	0.286	0.310
Immunohistochemistry, spleen			
CHI3L3	↑ 0.032*	n.d.	n.d.
RT-qPCR (mRNA), hippocampus			
<i>Il1α</i>	↑ 0.032*	n.d.	n.d.
<i>Il2</i>	0.518	n.d.	n.d.
<i>Il4</i>	1.000	n.d.	n.d.
<i>Il5</i>	0.161	n.d.	n.d.
<i>Il6</i>	0.531	n.d.	n.d.
<i>Il10</i>	0.600	n.d.	n.d.
<i>Ton</i>	0.064	n.d.	n.d.
<i>Tgfβ</i>	↑ 0.016*	n.d.	n.d.
<i>Ifny</i>	0.064	n.d.	n.d.
<i>Foxp3</i>	↑ 0.032*	n.d.	n.d.
<i>TMEV</i>	0.064	n.d.	n.d.
TMEV Plaque assay, cerebrum			
PFU/ml	0.905	n.d.	n.d.
RT-qPCR (mRNA), single samples, spleen			
<i>Chi3l3</i>	↑ 0.022*	n.d.	n.d.
<i>Ccl2</i>	0.070	n.d.	n.d.
<i>Ifi16</i>	0.263	n.d.	n.d.
Flow cytometry, blood			
CD4 ⁺ [%]	1.0000	n.d.	n.d.
CD8 ⁺ [%]	0.753	n.d.	n.d.
CD19 ⁺ [%]	1.000	n.d.	n.d.
CD4 ⁺ Foxp3 ⁺ [%]	0.835	n.d.	n.d.
gMFI CD69 gated on CD4 ⁺ cells	0.531	n.d.	n.d.
gMFI CD69 gated on CD8 ⁺ cells	0.531	n.d.	n.d.
gMFI CD44 gated on CD4 ⁺ cells	0.676	n.d.	n.d.
gMFI CD44 gated on CD8 ⁺ cells	1.000	n.d.	n.d.

Supplemental table S3: continued

Summary of statistical analyzes: IL-10R blockade during Theiler's murine encephalomyelitis virus (TMEV) infection at 7 days post infection; p-values			
Investigation	TMEV-infected SJL mice with and without IL-10R blockade	TMEV-infected C57BL/6 and SJL mice	TMEV-infected C57BL/6 mice and SJL mice with IL-10R blockade
Flow cytometry, spleen			
CD4 ⁺ [%]	0.210	n.d.	n.d.
CD8 ⁺ [%]	0.403	n.d.	n.d.
CD19 ⁺ [%]	0.296	n.d.	n.d.
CD4 ⁺ Foxp3 ⁺ [%]	1.000	n.d.	n.d.
gMFI CD69 gated on CD4 ⁺ cells	0.144	n.d.	n.d.
gMFI CD69 gated on CD8 ⁺ cells	0.210	n.d.	n.d.
gMFI CD44 gated on CD4 ⁺ cells	0.144	n.d.	n.d.
gMFI CD44 gated on CD8 ⁺ cells	↑ 0.037*	n.d.	n.d.

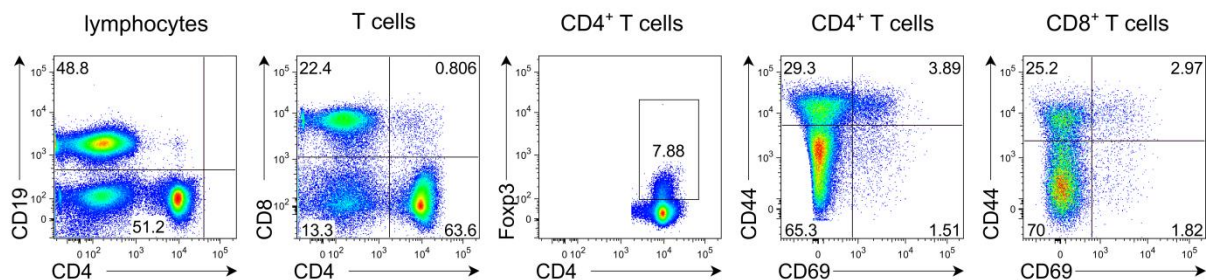
↑↓ = significant up(↑)- or downregulation (↓) in first versus the second group; * bold p-values = significant difference between both groups, $p \leq 0.05$, Mann-Whitney U tests. β -APP = β -amyloid precursor protein; PFU = Plaque forming units; gMFI = geometric mean of fluorescence intensity; H&E = hematoxylin and eosin staining; NeuN = neuron-specific nuclear protein; n.d.= not determined

Supplemental figures

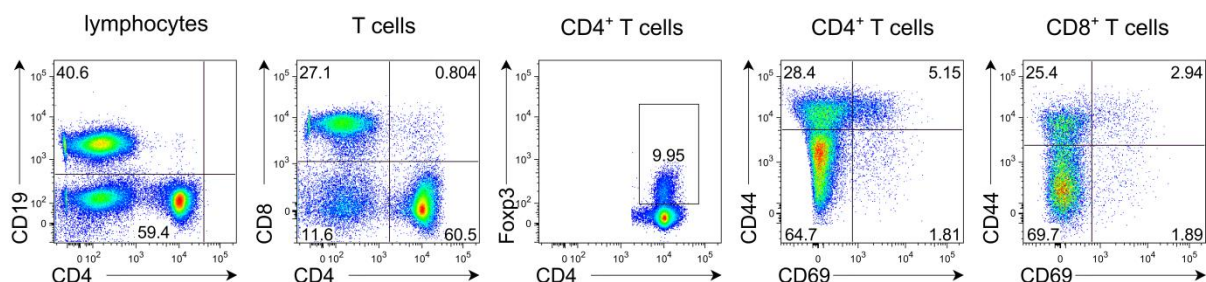


Supplemental figure S1: quantification of Theiler's murine encephalomyelitis virus (TMEV) in the brain. (a) Quantification of viral RNA by RT-qPCR. (b) Quantification of infectious virus by plaque assay. Statistical analyzes reveal a slight, non-significant increase of TMEV RNA in animals with IL-10R blockade compared to TMEV-infected isotype-treated animals at 7 days post infection. However, no differences are detected in the quantification of infectious virus particles. Box plots display median and quartiles with minimum and maximum values. PFU = Plaque forming units.

a SJL/isotype/TMEV



b SJL/IL-10R $\downarrow$ /TMEV



Supplemental figure S2: Flow cytometry analysis of Theiler's murine encephalomyelitis virus (TMEV)-infected SJL mice following IL-10R blockade at 7 days post infection. Representative dot plots of an isotype-treated animal (control) (a) and an IL-10R-blocked animal (b). No significant differences were detected in the percentages of investigated cell subsets including CD19⁺ B cells, CD4⁺ T cells and CD8⁺ T cells, as well as expression of CD44 and CD69 on CD4⁺ T cells and CD8⁺ T cells, respectively.