

Systemic inhibition of the membrane attack complex impedes neuroinflammation in chronic relapsing experimental autoimmune encephalomyelitis

Iliana Michailidou^{1,2}, Aldo Jongejan³, Jeroen P. Vreijling², Theodosia Georgakopoulou¹, Marit B. de Wissel¹, Ruud A. Wolterman¹, Patrick Ruizendaal¹, Ngaisah Klar-Mohamad⁴, Anita E. Grootemaat⁵, Daisy I. Picavet⁵, Vinod Kumar⁶, Cees van Kooten⁴, Trent M. Woodruff⁶, B. Paul Morgan⁷, Nicole N. van der Wel⁵, Valeria Ramaglia^{1,8}, Kees Fluiter^{2†} and Frank Baas^{2†*}

¹Department of Genome Analysis, Academic Medical Center, Amsterdam, The Netherlands; ²Department of Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands, ³Department of Bioinformatics, Academic Medical Center, Amsterdam, The Netherlands; ⁴Department of Nephrology, Leiden University Medical Center, Leiden, The Netherlands; ⁵Electron Microscopy Centre Amsterdam, Department of Medical Biology, Academic Medical Center, Amsterdam, The Netherlands; ⁶School of Biomedical Sciences, The University of Queensland, Brisbane, Australia; ⁷Systems Immunity University Research Institute, School of Medicine, Cardiff University, Cardiff, UK; ⁸Department of Immunology, University of Toronto, Toronto, Canada.

[†] authors contributed equally to this study.

*Corresponding author: Prof Dr Frank Baas, Department of Clinical Genetics, Leiden University Medical Center, Einthovenweg 20, 2333 ZC Leiden, The Netherlands. Tel: 0031 71 526 9868. E-mail: F.Baas@lumc.nl

Table S1 Expression levels of key immune genes in mice with chronic relapsing EAE showing mild or severe neurological disability and comparison with mice showing no disability

GenBank ID	Symbol	Description / Full name	Av. Express. No Disability	Av. Express. Mild Disability	Av. Express. Severe Disability
<i>Complement System</i>					
NM_009778	C3	complement component 3	0.113	0.152, <i>p</i> =ns	0.843, ***<i>p</i><0.001
NM_016704	C6	complement component 6	0.154	0.256, <i>p</i> =ns	1.247, ***<i>p</i><0.001
NM_010016	Cd55	decay accelerating factor for complement	1.549	1.523, <i>p</i> =ns	1.465, <i>p</i> =ns
NM_009776	Serping1 (C1INH)	serine (or cysteine) peptidase inhibitor, clade G, member 1	0.163	0.201, <i>p</i> =ns	1.104, ***<i>p</i><0.001
<i>Inflammasomes</i>					
NM_001004142	Nlrp1a	NLR family, pyrin domain containing 1A	0.079	0.078, <i>p</i> =ns	0.965, ***<i>p</i><0.001
NM_145827	Nlrp3	NLR family, pyrin domain containing 3	0.055	0.071, <i>p</i> =ns	0.834, ***<i>p</i><0.001
NM_001013779	Aim2	absent in melanoma 2	0.513	1.406, * <i>p</i> =0.05	0.825, <i>p</i> =ns
NM_023258	Pycard (Asc)	PYD and CARD domain containing	0.241	0.236, <i>p</i> =ns	0.885, ***<i>p</i><0.001
NM_001033367	Nlrc4	NLR family, CARD domain containing 4	0.049	0.050, <i>p</i> =ns	0.872, ***<i>p</i><0.001
NM_008361	Il1β	interleukin 1 beta	0.006	0.007, <i>p</i> =ns	0.601, ***<i>p</i><0.001
<i>Monocyte/Macrophage activation</i>					
NM_031195	Msr1	macrophage scavenger receptor 1	0.006	0.007, <i>p</i> =ns	0.527, ***<i>p</i><0.001
NM_009841	Cd14	antigen expressed in	0.025	0.029, <i>p</i> =ns	0.681, ***<i>p</i><0.001

		monocyte/macrophages			
NM_007643	Cd36	antigen expressed in	0.026	0.018, <i>p</i> =ns	0.852, ***<i>p</i><0.01
		monocyte/macrophages			
NM_011905	Tlr2	toll-like receptor 2	0.037	0.044, <i>p</i> =ns	0.790, ***<i>p</i><0.001
NM_021297	Tlr4	toll-like receptor 4	0.051	0.062, <i>p</i> =ns	0.754, ***<i>p</i><0.001
NM_031168	Il6	interleukin 6	0.012	0.012, <i>p</i> =ns	0.644, ***<i>p</i><0.001
NM_008689	NFκB1	nuclear transcription	0.223	0.238, <i>p</i> =ns	0.856, ***<i>p</i><0.001
		factor			
NM_011333	Ccl2	Ccl2 chemokine	0.005	0.005, <i>p</i> =ns	0.728, **<i>p</i><0.01
		(C-C motif) ligand 2			
<i>Astroglia activation</i>					
NM_001131020	Gfap1	glial fibrillary acidic	0.360	0.419, <i>p</i> =ns	0.849, ***<i>p</i><0.001
		protein			
<i>LXR/RXR pathway</i>					
NM_013839	Nr1h3	nuclear receptor subfamily	0.297	0.330, <i>p</i> =ns	0.892, ***<i>p</i><0.001
	(lxra)	1, group H, member 3			
NM_009473	Nr1h2	nuclear receptor subfamily	0.766	0.843, <i>p</i> =ns	1.022, ***<i>p</i><0.001
	(lxrb)	1, group H, member 2			
NM_013454	Abca1	ATP-binding cassette, sub-	0.096	0.098, <i>p</i> =ns	0.582, ***<i>p</i><0.001
		family A (ABC1), member			
		1			
NM_009696	Apoe	apolipoprotein E	0.207	0.268, <i>p</i> =ns	0.821, ***<i>p</i><0.001
NM_011305	Rxra	retinoid X receptor alpha	0.513	0.604, <i>p</i> =ns	0.781, ***<i>p</i><0.001
NM_009107	Rxrg	retinoid X receptor gamma	1.336	1.458, <i>p</i> =ns	1.237, <i>p</i> =ns

No Disability: clinical score=0; Mild Disability: 0<clinical score≤3; Severe Disability: 3<clinical score≤5. Statistically significant values are marked in bold. *ns*: non-significant

Table S2 Mouse primer sequences

Target gene	Primer	Sequence
C3	Forward	accttacctcggcaagtttct
	Reverse	ttgtagagctgctggtcagg
C6	Forward	cagagaaaaatgaacattcccatta
	Reverse	ttcttgagggaagctttaatgac
Cd55	Forward	actgttgattgggacgatgag
	Reverse	tggtggctctggacaatgta
Serping1 (C1INH)	Forward	ccaaaggtgtcacttctgtgtc
	Reverse	gagatgcattcacataggtgtcc
Nlrp1a	Forward	agacgttgccccatggt
	Reverse	ggtgggtcaggctgtctcta
Nlrp3	Forward	gaattccggccttacttcaa
	Reverse	ggtgtgtgaagttctggttg
Aim2	Forward	tgggctgtttaagtccagaa
	Reverse	cacctcattgtccctgttt
Pycard (Asc)	Forward	ccagagttctgtttcttaccttgag
	Reverse	tcacagcccagagtttagatgc
Nlrc4	Forward	ctgcaagaacatgcataccc
	Reverse	tactgttcgtcatccgtggt
Il1 β	Forward	agttgacggaccccaaaag
	Reverse	agctggatgctctcatcagg
Msr1	Forward	ctggacaaactggtccacct
	Reverse	gtccccgatcaccttaaca
Cd14	Forward	aaagaaactgaagcctttctcg
	Reverse	agcaacaagccaagcacac
Cd36	Forward	tgaaaagtctcggacattgag
	Reverse	tcagatccgaacacagcgta
Tlr2	Forward	ggggcttcacttctctgctt
	Reverse	agcatcctctgagatttgacg
Tlr4	Forward	ggactctgatcatggcactg
	Reverse	ctgatccatgcattggttaggt

Il6	Forward	gctaccaaactggatataatcagga
	Reverse	ccaggtagctatggtactccagaa
NFkβ1	Forward	ctgctcaggtccactgtctg
	Reverse	tgtcactatcccggagttca
Ccl2	Forward	catccacgtgttggtca
	Reverse	gatcatcttgctggtgaatgagt
Gfap1	Forward	cagcttacggccaacagtg
	Reverse	gcctcaggttggtttcatct
Nr1h3 (lxra)	Forward	gagtgtcgacttcgaaatg
	Reverse	cggatctgttcttctgacagc
Nr1h2 (lxrb)	Forward	tgccagggttcttcagtg
	Reverse	agcgtctggctgtttctagc
Abca1	Forward	ttcatcctcctgtcatctc
	Reverse	tccttggcaaagttcacaaa
ApoE	Forward	ttggtcacattgctgacagg
	Reverse	agcgcaggtaatccagaa
Rxra	Forward	acatgcagatggacaagacg
	Reverse	gggtttgagagccccttaga
Rxrg	Forward	cagaagtgcctgggtcatgg
	Reverse	cctcactctgctcgctct
HPRT	Forward	ggtcattcctatgactgtagattt
	Reverse	caatcaagacgttctttccagtt

Table S3 Primary antibodies, dilution, source

Antigen	Clone	Dilution/Concentration	Source
PLP	plpc1	0.3µg/ml ^a	Serotec
MAG	Monoclonal (#ab89780)	1:1,000 ^c	Abcam
IBA-1	Polyclonal	1:3,000 ^c	Wako
CD3	SP7	1:500 ^b	Abcam
C9	Polyclonal	1:200 ^c	Made in house (BPM, Cardiff)
SYP	YE269	1:600 ^b	Abcam
SYP	SVP-38	1:100 ^b	Sigma
IL-1β/pro-IL-1β	Polyclonal	1:70	Santa Cruz
NLRP3	Polyclonal	1:100 ^b	Abcam

Antigen retrieval of paraffin sections was performed by heat in ^a 0.05 M Tris buffered saline pH 7.6; ^b 10 mM citric acid buffer pH 6.0; ^c 10 mM Tris/1 mM EDTA buffer pH 9.0.

Table S4 Log fold change values of NLRP3 inflammasome genes in the mouse spinal cord at the relapse of chronic relapsing EAE

Gene	no drug vs control	C6 antisense vs control	PMX205 vs control
CASP1	15,319	0,666	12,685
IL-1 β	19,370	-0,865	14,348
MYD88	18,952	0,873	0,731
NAIP	18,796	-0,162	0,906
NLRC4	12,786	0,576	0,698
NLRP1	15,981	-0,831	0,165
NLRP3	22,853	-0,484	15,752
PYCARD	1,349	-0,802	-0,181
TLR4	15,428	0,379	11,011

no drug: non-treated mice with chronic relapsing EAE; C6 antisense: mice with chronic relapsing EAE treated with the C6 antisense oligonucleotide; PMX205: mice with chronic relapsing EAE treated with the PMX205 antagonist of C5aR1; control: healthy naïve mice.