Supplemental Materials for

Membrane assembly of aquaporin-4 autoantibodies regulates classical complement activation in neuromyelitis optica

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The Supplemental file includes:

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Table S1: Binding affinity of AQP4 recombinant antibodies (rAb) with Fc domain mutations

AQP4 rAb	His151/Leu154 Dependence	Fc Domain	K_D (nM)	St Dev
ON 07-5 #53	No	IgG1	14.8	2.4
		AEFTE	14.2	5.1
		E345R	14.3	1.1
		I253D	14.1	1.1
		AEFTE/E345R	13.9	1.2
		AEFTE/I253D	14.3	2.9
		K322A	15.4	0.6
ON 07-5 #186	Yes	IgG1	15.2	2.4
		AEFTE	13.5	1.1
		E345R	12.6	4.6
		I253D	18.7	1.3
		AEFTE/E345R	12.9	1.1
		AEFTE/I253D	17.6	2.7

[A] His151/Leu154 Dependence = binding dependence to the unique extracellular loop C amino acids His151 and Leu154

[B] K_D = equilibrium dissociation constant for rAb binding to AQP4

[C] AEFTE = G236$\underline{A}$/S267$\underline{E}$/H268$\underline{F}$/S324$\underline{T}$/I332$\underline{E}$ Fc domain mutations

[D] All measurements represent ≥3 experimental replicates

Table S2: Complement-dependent cytotoxicity (CDC) and C1q binding by AQP4 recombinant antibody (rAb) with Fc domain mutations

AQP4 rAb	His151/Leu154 Dependence	Fc Domain	CDC EC50 (nM)	St Error	C1q/AQP4 Bmax	St Error	C1q/AQP4 at EC50	% of C1q/AQP4 B_{MAX} at CDC EC50
ON 07-5 #53	No	IgG1	20.1	2.6	19.6	2.0	10.5	53.6
		AEFTE	9.3	1.5	109.4	4.5	30.7	28.1
		E345R	4.1	2.1	104.3	8.7	7.9	7.6
		I253D	NA	NA	3.5	1.3	-	-
		AEFTE/E345R	4.2	1.5	102.7	3.0	6.7	6.5
		AEFTE/I253D	NA	NA	2.1	2.2	-	-
		K322A	NA	NA	3.0	2.2	-	-
ON 07-5 #58	No	IgG1	51.43	4.3	11.1	0.9	8.8	79.3
		AEFTE	8.4	1.7	47.3	2.8	4.5	9.5
		E345R	2.6	0.3	48.4	5.1	4.6	9.5
		I253D	NA	NA	7.9	3.6	-	-
ON 10-1 #121	No	IgG1	1226.0	199.3	17.6	11.8	-	-
		AEFTE	32.4	4.2	96.7	17.2	17.4	18.0
		E345R	63.2	4.7	58.2	21.8	24.2	41.6
		I253D	NA	NA	-	-	-	-
ON 09-3 #33	Yes	IgG1	10.8	1.2	8.6	1.0	5.7	66.3
		AEFTE	2.3	1.2	75.4	5.1	7.26	9.6
		E345R	3.8	1.6	70.0	6.9	29.7	42.4
		K322A	NA	NA	0.8	0.4	-	-
		I253D	NA	NA	6.0	1.2	-	-
ON 07-5 #93	Yes	IgG1	10.9	1	53.0	6.4	8.2	15.5
		AEFTE	5.8	0.5	79.6	2.9	11.2	14.1
		E345R	8.9	0.7	83.6	3.5	16.5	19.7
		I253D	112.8	8.6	16.2	1.6	14.7	90.7
ON 10-1 #153	Yes	IgG1	16.6	0.9	9.9	1.3	2.7	27.3
		AEFTE	3.4	0.4	94.7	5.1	5.4	5.7
		E345R	6.2	0.9	94.2	6.8	12.7	13.5
		I253D	NA	NA	2.3	0.5	-	-
ON 07-5 #186	Yes	IgG1	3.2	1.5	89.8	16.5	5.2	5.8
		AEFTE	2.6	2.1	161.9	12.1	27.6	17.0
		E345R	4.4	0.4	169.3	18.8	20.3	12.0
		I253D	NA	NA	2.8	1.0	-	-
		AEFTE/E345R	4.2	0.3	110.2	3.4	15.5	14.1
		AEFTE/I253D	NA	NA	2.9	0.5	-	-

[A] His151/Leu154 Dependence = binding dependence to the unique extracellular loop C amino acids His151 and Leu154

[B] AEFTE = G236A/S267E/H268F/S324T/I332E Fc domain mutations

[C] EC50 = effective concentration at which 50% cell lysis occurs by CDC using 5% human complement

[D] NA = No activation of CDC

[E] C1q/AQP4 = ratio of C1q fluorescence intensity over total AQP4 fluorescence intensity

[F] C1q/AQP4 B_{MAX} = maximum level of C1q bound at saturating C1q levels

[G] All measurements represent ≥3 experimental replicates

Table S3: C1q binding to AQP4 recombinant antibodies (rAb) over M1-AQP4

AQP4 rAb	H151/L154-Dependence	Fc Domain	[rAb] (nM) when C1q levels surpass level at CDC EC50 over M23-AQP4	Maximum Observed C1q/AQP4 ± St Error
ON 07-5 #53	No	IgG1	-	5.7 ± 0.6
		AEFTE	243	17.4 ± 2.8
		E345R	-	5.0 ± 0.5
		K322A	-	0.0 ± 0.8
ON 07-5 #58	No	IgG1	-	5.9 ± 2.3
		AEFTE	60	38.7 ± 4.4
		E345R	-	5.9 ± 0.6
ON 10-1 #153	Yes	IgG1	156	6.9 ± 2.2
		AEFTE	76	21.9 ± 0.3
		E345R	178	6.4 ± 0.2
ON 07-5 #186	Yes	IgG1	254	8.3 ± 0.6
		AEFTE	131	26.8 ± 3.6
		E345R	249	9.7 ± 0.6

[A] H151/L154 Dependence = binding dependence to the unique extracellular loop C amino acids His151 and Leu154

[B] AEFTE = G236A/S267E/H268F/S324T/I332E Fc domain mutations

[C] CDC EC50 = effective concentration at which 50% cell lysis occurs by complement-dependent cytotoxicity using 5% human complement

[D] C1q/AQP4 = ratio of C1q fluorescence intensity over total AQP4 fluorescence intensity