

Supporting Information for

Dissection of complement and Fc-receptor mediated pathomechanisms of autoantibodies to myelin oligodendrocyte glycoprotein.

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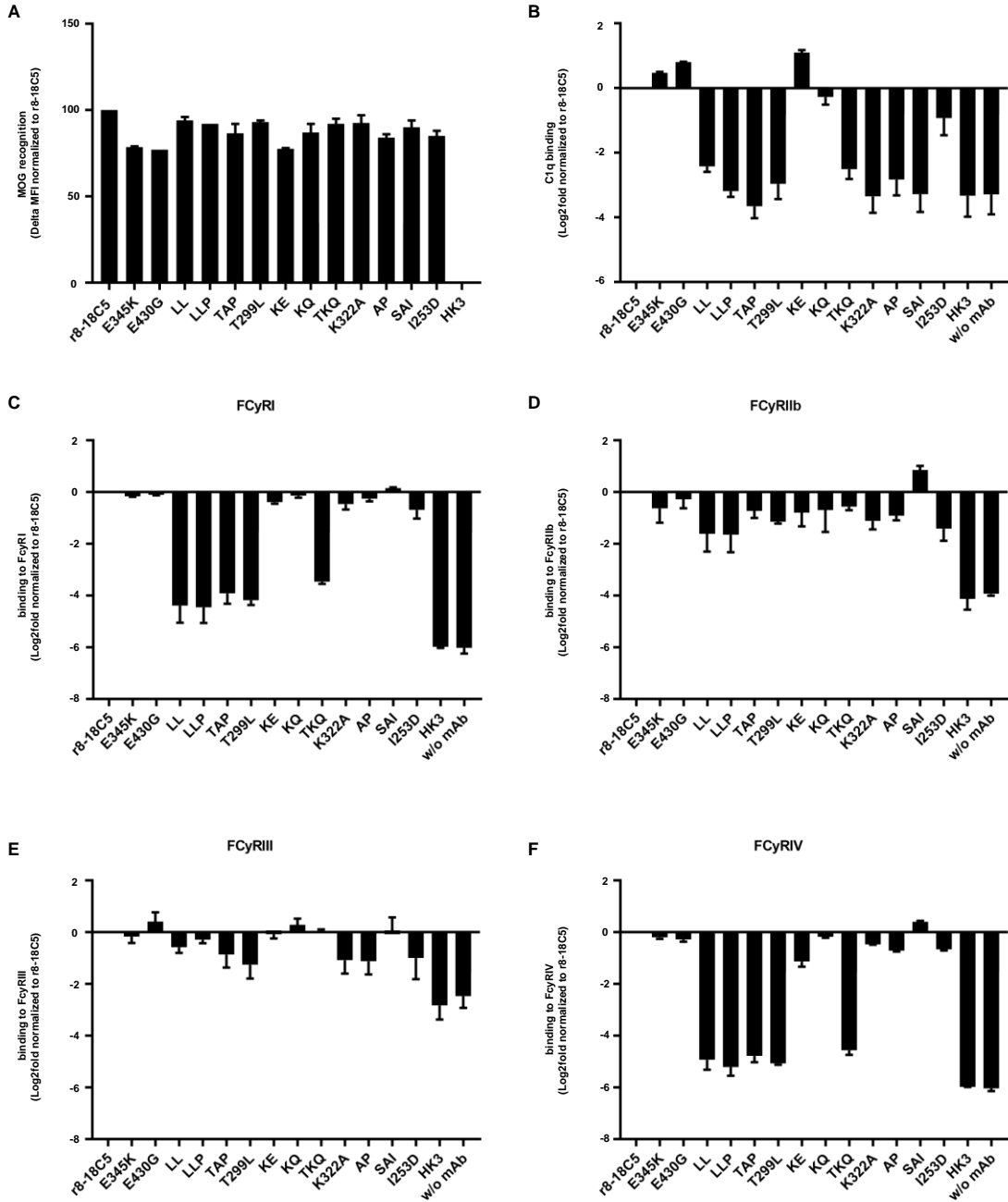
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Supplementary figure



Supplementary Figure 1: Antigen-recognition, C1q- and FcγR-binding of mutated MOG-specific mAbs.

The antibody r8-18C5 with human IgG1 Fc part and all 13 Fc mutant antibodies show comparable binding to MOG in a cell based flow cytometry assay (A). IgG binding to

MOG was normalized to binding of the r8-18C5 (delta MFI value). The assay was repeated twice for each antibody (**B**) C1q binding was analyzed by ELISA. Specifically, ED-hMOG was coated first before the addition of the indicated mAbs. C1q was added and its binding was quantified using an anti-C1q Ab. Binding of the mutant Abs was normalized to the binding of the antibody r8-18C5 and the assay was repeated three times for each antibody. (**C-F**) Fc γ R binding was investigated by ELISA. Anti-FLAG antibodies were coated on an ELISA plate, which bound to the soluble Fc γ R (Fc γ RI, Fc γ RIIb, Fc γ RIII, Fc γ RIV). We quantified the binding of antibody-MOG complexes (immune complexes) to the soluble Fc γ R. Binding of the mutant Abs was normalized to the binding of the antibody r8-18C5. Fc γ RI and Fc γ RIV were read 40 min after reaction started and Fc γ RIIb, Fc γ RIII were read 300 min after reaction started. The assay was repeated three times for the antibodies that were used *in vivo* (HK3, TAP, SAI and r8-18C5), while it was performed twice for all other antibodies. Data is shown as mean with SEM.

Table S1. Fc mutants of the MOG mAb r8-18C5 used in this study

Variant (name used in our manuscript)	Introduced mutation	Previously observed effect with other mAbs	Observed effect after MOG-binding
E345K	E345K	enhanced C1q binding/ CDC ¹ enhanced FcyR binding/ ADCC ^{1,2}	intact CDC intact FcyR binding
E430G	E430G	enhanced C1q binding/CDC ¹ enhanced FcyR binding/ ADCC ²	intact CDC intact FcyR binding
LL	L234A/L235A	reduced C1q binding/ CDC ^{3,4} reduced FcyR binding/ ADCC ^{3,4}	reduced C1q reduced FcyR binding
LLP	L234A/L235A/P329G	reduced C1q binding/CDC ⁵ reduced FcyR binding/ ADCC ⁵	reduced C1q reduced FcyR binding
TAP	T299L/A330S/P331S	reduced C1q binding/CDC ⁶⁻⁸ not defined for FcyR binding/ ADCC	reduced C1q reduced FcyR binding
T299L	T299L	not defined for C1q binding/CDC reduced FcyR binding/ ADCC ⁹	reduced C1q reduced FcyR binding
KE	K326W/E333S	enhanced C1q binding/CDC ^{10,11} reduced FcyR binding/ ADCC ¹⁰	intact CDC intact FcyR binding
KQ	K320E/Q386R	enhanced C1q binding/CDC ⁹ reduced FcyR binding/ ADCC ⁹	intact CDC intact FcyR binding
TKQ	T299L/K320E/Q386R	enhanced C1q binding/CDC ⁹ intact FcyR binding/ ADCC ⁹	reduced C1q reduced FcyR binding
K322A	K322A	reduced C1q binding/CDC ¹² intact FcyR binding/ ADCC ⁴	reduced C1q intact FcyR binding
AP	A330S/P331S	reduced C1q binding/CDC ⁶ reduced FcyR binding/ ADCC ¹³	reduced C1q intact FcyR binding
SAI	S239D/A330L/I332E	reduced C1q binding/CDC ^{7,10} enhanced FcyR binding/ ADCC ^{7,10,14}	reduced C1q intact FcyR binding
I253D	I253D	reduced C1q binding/CDC ^{12,15} not defined for FcyR binding/ ADCC	reduced CDC intact FcyR binding

SI References

1. Canfield SM, Morrison SL. The binding affinity of human IgG for its high affinity Fc receptor is determined by multiple amino acids in the CH2 domain and is modulated by the hinge region. *J Exp Med*. Jun 1 1991;173(6):1483-91. doi:10.1084/jem.173.6.1483
2. Gross JA, Dillon SR, Mudri S, et al. TACI-Ig neutralizes molecules critical for B cell development and autoimmune disease. impaired B cell maturation in mice lacking BLyS. *Immunity*. Aug 2001;15(2):289-302. doi:10.1016/s1074-7613(01)00183-2
3. Lee CH, Romain G, Yan W, et al. Corrigendum: IgG Fc domains that bind C1q but not effector Fc gamma receptors delineate the importance of complement-mediated effector functions. *Nat Immunol*. Sep 19 2017;18(10):1173. doi:10.1038/ni1017-1173c
4. Idusogie EE, Wong PY, Presta LG, et al. Engineered antibodies with increased activity to recruit complement. *J Immunol*. Feb 15 2001;166(4):2571-5. doi:10.4049/jimmunol.166.4.2571
5. Armour KL, Clark MR, Hadley AG, Williamson LM. Recombinant human IgG molecules lacking Fc gamma receptor I binding and monocyte triggering activities. *Eur J Immunol*. Aug 1999;29(8):2613-24. doi:10.1002/(SICI)1521-4141(199908)29:08<2613::AID-IMMU2613>3.0.CO;2-J
6. Liu R, Oldham RJ, Teal E, Beers SA, Cragg MS. Fc-Engineering for Modulated Effector Functions-Improving Antibodies for Cancer Treatment. *Antibodies (Basel)*. Nov 17 2020;9(4)doi:10.3390/antib9040064
7. Lazar GA, Dang W, Karki S, et al. Engineered antibody Fc variants with enhanced effector function. *Proc Natl Acad Sci U S A*. Mar 14 2006;103(11):4005-10. doi:10.1073/pnas.0508123103
8. Lee CH, Romain G, Yan W, et al. IgG Fc domains that bind C1q but not effector Fc gamma receptors delineate the importance of complement-mediated effector functions. *Nat Immunol*. Aug 2017;18(8):889-898. doi:10.1038/ni.3770
9. Bondza S, Marosan A, Kara S, et al. Complement-Dependent Activity of CD20-Specific IgG Correlates With Bivalent Antigen Binding and C1q Binding Strength. *Front Immunol*. 2020;11:609941. doi:10.3389/fimmu.2020.609941
10. Ratelade J, Asavapanumas N, Ritchie AM, Wemlinger S, Bennett JL, Verkman AS. Involvement of antibody-dependent cell-mediated cytotoxicity in inflammatory demyelination in a mouse model of neuromyelitis optica. *Acta Neuropathol*. Nov 2013;126(5):699-709. doi:10.1007/s00401-013-1172-z
11. Jarius S, Metz I, Konig FB, et al. Screening for MOG-IgG and 27 other anti-glial and anti-neuronal autoantibodies in 'pattern II multiple sclerosis' and brain biopsy findings in a MOG-IgG-positive case. *Mult Scler*. Oct 2016;22(12):1541-1549. doi:10.1177/1352458515622986
12. Soltys J, Liu Y, Ritchie A, et al. Membrane assembly of aquaporin-4 autoantibodies regulates classical complement activation in neuromyelitis optica. *J Clin Invest*. Apr 8 2019;129(5):2000-2013. doi:10.1172/JCI122942
13. Lund J, Winter G, Jones PT, et al. Human Fc gamma RI and Fc gamma RII interact with distinct but overlapping sites on human IgG. *J Immunol*. Oct 15 1991;147(8):2657-62.
14. Xu H, Guo H, Cheung IY, Cheung NK. Antitumor Efficacy of Anti-GD2 IgG1 Is Enhanced by Fc Glyco-Engineering. *Cancer Immunol Res*. Jul 2016;4(7):631-8. doi:10.1158/2326-6066.CIR-15-0221
15. Diebolder CA, Beurskens FJ, de Jong RN, et al. Complement is activated by IgG hexamers assembled at the cell surface. *Science*. Mar 14 2014;343(6176):1260-3. doi:10.1126/science.1248943