**Supplementary Materials**

**Distinct impact of IgG subclass on autoantibody pathogenicity in different IgG4-mediated diseases**

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**Supplementary Tables:**

**Supplementary file 1a. Affinities of mouse FcγRs for human IgG4, IgG1 and its variants.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|   | FcγRI | FcγRIIB | FcγRIII | FcγRIV | Reference paper |
| IgG1 | 1(0.15μM) | 1(9.92μM) | 1(30.06μM) | 1(0.41μM) | a-d |
| IgG4 | 0.62-0.80 | < 0.90 | < 1.43 | < 0.02 | a,c |
| GASDALIE | 0.19-1.52 | 3.54-76.28 | 7.16-3005.75 | 2.07-413.00 | b,d |
| N297A | n. d. | n.d. | n.d. | n.d.b. | e |

n.d.b. indicates no detectable binding. n.d. indicates not done.

Reference paper:

a: Derebe, M. G., Nanjunda, R. K., Gilliland, G. L., Lacy, E. R., & Chiu, M. L. (2018). Human IgG subclass cross-species reactivity to mouse and cynomolgus monkey Fcgamma receptors. *Immunol Lett, 197*, 1-8. doi:10.1016/j.imlet.2018.02.006

b: Bournazos, S., Klein, F., Pietzsch, J., Seaman, M. S., Nussenzweig, M. C., & Ravetch, J. V. (2014). Broadly neutralizing anti-HIV-1 antibodies require Fc effector functions for in vivo activity. *Cell, 158*(6), 1243-1253. doi:10.1016/j.cell.2014.08.023

c: Dekkers, G., Bentlage, A. E. H., Stegmann, T. C., Howie, H. L., Lissenberg-Thunnissen, S., Zimring, J., . . . Vidarsson, G. (2017). Affinity of human IgG subclasses to mouse Fc gamma receptors. *MAbs, 9*(5), 767-773. doi:10.1080/19420862.2017.1323159

d: Gunn, B. M., Lu, R., Slein, M. D., Ilinykh, P. A., Huang, K., Atyeo, C., . . . Alter, G. (2021). A Fc engineering approach to define functional humoral correlates of immunity against Ebola virus. *Immunity, 54*(4), 815-828 e815. doi:10.1016/j.immuni.2021.03.009

e: Nimmerjahn, F., Bruhns, P., Horiuchi, K., & Ravetch, J. V. (2005). FcgammaRIV: a novel FcR with distinct IgG subclass specificity. *Immunity, 23*(1), 41-51. doi:10.1016/j.immuni.2005.05.010

**Supplementary file 1b. FcγR binding properties of human IgG1 and its variants (f, g)**

|  |  |  |  |
| --- | --- | --- | --- |
| IgG1&variants | Fc mutation | Activating | Inhibitory |
| FcγRIIa | FcγRIIIa | FcγRIIb |
| IgG1 | - | + | + | + |
| GASDALIE | G236A/S239D/A330L/I332E | +++ | +++ | + |
| N297A | N297A | - | - | - |

f: Bournazos, S., DiLillo, D. J., Goff, A. J., Glass, P. J., & Ravetch, J. V. (2019). Differential requirements for FcgammaR engagement by protective antibodies against Ebola virus. *Proc Natl Acad Sci U S A, 116*(40), 20054-20062. doi:10.1073/pnas.1911842116

g: Sazinsky, S. L., Ott, R. G., Silver, N. W., Tidor, B., Ravetch, J. V., & Wittrup, K. D. (2008). Aglycosylated immunoglobulin G1 variants productively engage activating Fc receptors. *Proc Natl Acad Sci U S A, 105*(51), 20167-20172. doi:10.1073/pnas.0809257105

**Supplementary file 1c. Demographic characteristics and laboratory findings of TTP patients**

|  |  |
| --- | --- |
| Sex, F/M | 22/21/1A |
| Median age, years (range) | 57 (5-83) |
| ADAMTS13 Ac (< 5%) /n | 44/44 |
|  TTP | 1.3 (0.6-2.7)% |
|  HC | 79.2 (52-105)% |
| ADAMTS13 Ag (ng/ml)  |  |
|  TTP | 28 (0-178) |
|  HC | 487 (306-658) |
| IgG1 dominant TTP/n | 20/44 |
|  ADAMTS13 Ac% | 1.274 (0.808-1.781) |
|  ADAMTS13 Ag (ng/ml)  | 12.535 (0-101.572) |
| IgG4 dominant TTP/n | 24/44 |
|  ADAMTS13 Ac% | 1.384 (0.595-2.674) |
|  ADAMTS13 Ag (ng/ml)  | 40.953 (2.270-178.372) |

ABasic information of 1 TTP patients is incomplete.

**Supplementary file 1d. Demographic characteristics and laboratory findings of PF patients**

|  |  |
| --- | --- |
| Sex, F/M | 18/35 |
| Median age, years (range) | 62 (29-92) |
| αDsg1 unit value (U/ml) 1:100 | 191 (79-354) |
|  Active | 197 (79-315) |
|  Stable | 194 (101-354) |
| αDsg1 IgG unit value (U/ml) 1:1000 | 723 (260-2830) |
|  Active | 1039 (284-2830) |
|  Stable | 646 (260-1357) |
| αDsg1 IgG1 unit value (U/ml) 1:100 | 42 (2-188) |
|  Active | 62 (6-188) |
|  Stable | 35 (2-116) |
| αDsg1 IgG4 unit value (U/ml) 1:1000 | 538 (0-1338) |
|  Active | 633 (0-1338) |
|  Stable | 416 (8-1046) |

**Supplementary file 1e. Characteristics of anti-Dsg1 mAbs**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Clone name | Heavy chain | VL | IIF/human | Pathogenicity | Dsg1 epitope |
| V | D | J | human  | mouse |
| PF1-8-15 | VH3-30 | D5-24 | Jh4b | 3h | + | + | + | 89-101aa |
| PF24-9 | VH3-53 | D4 | Jh4b | 1c | + | + | + | 1-161aa |
| PF1-2-22 | VH1-08 | D3-3/DXP4 | Jh6b | O12/O2 | + | - | - | 1-161aa |

**Supplementary file 1f. PCR primers used for cloning Dsg1, Dsg2, and Dsg1/Dsg2 chimeric molecules**

|  |  |  |
| --- | --- | --- |
| Upstream fragments | Downstream fragments | Dsg1/Dsg2 chimeric molecules |
| PCR Products | Primers | PCR Products | Primers | PCR Products | Primers |
| F | R | F | R | F | R |
| Dsg1 | 1 | 2 | Dsg2 | 3 | 4 |  |  |  |
|  |  |   |  |  |   |  |  |  |
| Dsg1 EC1 | 1 | 5 | Dsg2 EC2-5 | 6 | 4 | EC1 | 1 | 4 |
| Dsg1 EC1-2 | 1 | 7 | Dsg2 EC3-5 | 8 | 4 | EC-2 | 1 | 4 |
| Dsg1 EC1-3 | 1 | 9 | Dsg2 EC4-5 | 10 | 4 | EC1-3 | 1 | 4 |
| Dsg1 EC1-4 | 1 | 11 | Dsg2 EC5 | 12 | 4 | EC1-4 | 1 | 4 |
|  |  |   |  |  |   |  |  |  |
| Dsg1 EC2-5 | 14 | 2 | Dsg2 EC1 | 3 | 13 | EC2-5 | 3 | 2 |
| Dsg1 EC3-5 | 16 | 2 | Dsg2 EC1-2 | 3 | 15 | EC-3-5 | 3 | 2 |
| Dsg1 EC4-5 | 18 | 2 | Dsg2 EC1-3 | 3 | 17 | EC4-5 | 3 | 2 |
| Dsg1 EC5 | 20 | 2 | Dsg2 EC1-4 | 3 | 19 | EC5 | 3 | 2 |
| Primer 1 | 5'-TTTGGATCCATGGACTGGAGTTTCTTCAGAGTAGTT |
| Primer 2 | 5'-TTTGTCGACATGTACATTGTCTGATAACAAATCTTTGG |
| Primer 3  | 5'-TTTGGATCCATGGCGCGGAGCCCGGGA |
| Primer 4  | 5'-TTTGTCGACGCCCACATAGGAGTCATGCTGTGCTTCC |
| Primer 5 | 5'-AACAAAGACATCCTGTGTAAACACTGGAGGGTTGTC |
| Primer 6  | 5'-GACAACCCTCCAGTGTTTACACAGGATGTCTTTGTT |
| Primer 7 | 5'-TTCAAGCACTTTATTTTCCATGTAAGGGATATTATC |
| Primer 8 | 5'-GATAATATCCCTTACATGGAAAATAAAGTGCTTGAA |
| Primer 9 | 5'-TGAGATGACGCTGCTTTTAAACACTGGGCCTTCAAT  |
| Primer 10 | 5'-ATTGAAGGCCCAGTGTTTAAAAGCAGCGTCATCTCA |
| Primer 11 | 5'-TGCATCGTGACAGATTGTAGTGTTCGGCTCTGTATT |
| Primer 12  | 5'-AATACAGAGCCGAACACTACAATCTGTCACGATGCA |
| Primer 13 | 5'-TGCAAATGTAGCCATTGAGAACACTGGTTCGTTGTC |
| Primer 14 | 5'-GACAACGAACCAGTGTTCTCAATGGCTACATTTGCA |
| Primer 15 | 5'-GGTATATGAAGACTGTTCTACTACAGGTATATTGTC |
| Primer 16 | 5'-GACAATATACCTGTAGTAGAACAGTCTTCATATACC |
| Primer 17 | 5'-TGTCTTTGAACCTGGACGAAAATGAATGCCTTCTTT |
| Primer 18 | 5'-AAAGAAGGCATTCATTTTCGTCCAGGTTCAAAGACA |
| Primer 19  | 5'-AGTATTGGTAGTAATTTTCTGCACAGGCTCTATCAG |
| Primer 20 | 5'-CTGATAGAGCCTGTGCAGAAAATTACTACCAATACT |