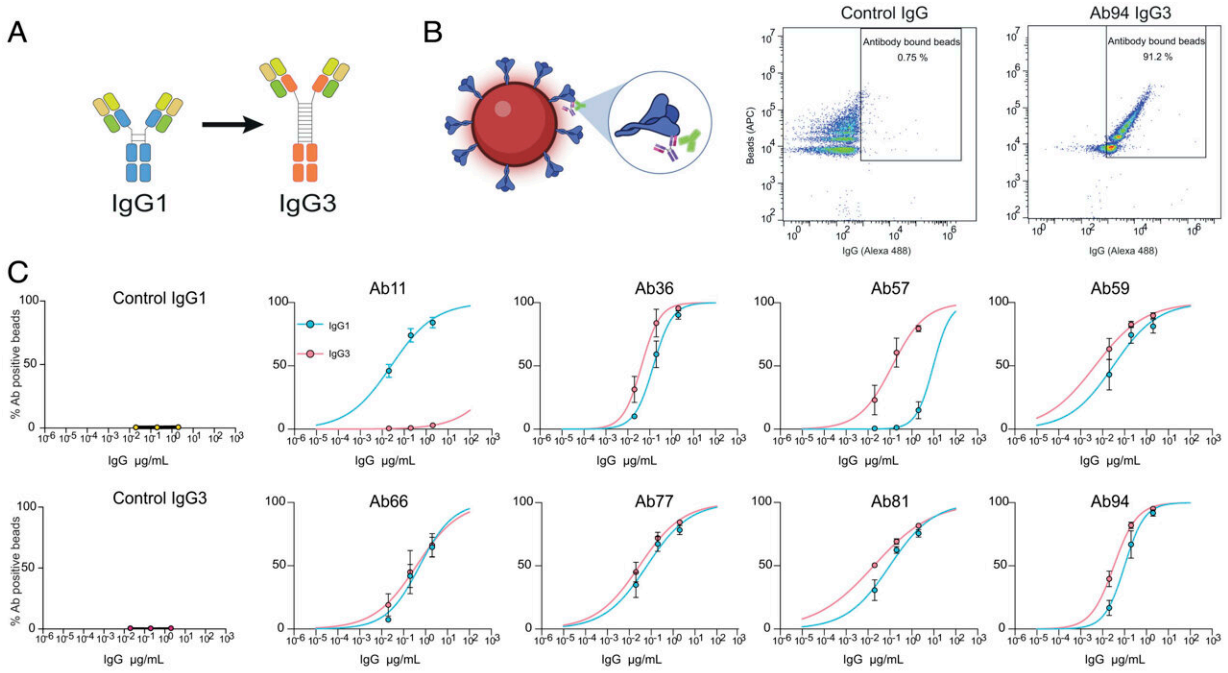
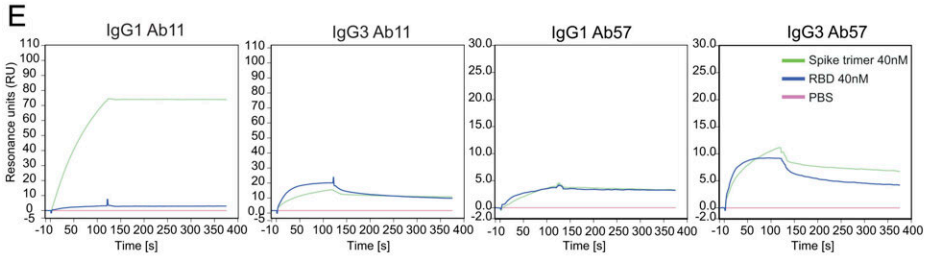
Cocktail of modified antibodies found to provide strong effect against SARS-CoV-2

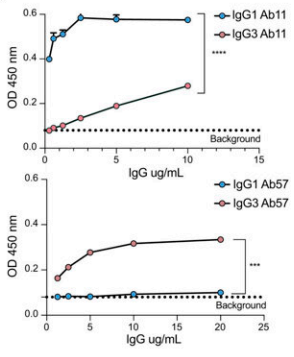
April 18 2023



D

Antibody	11	36	57	59	66	77	81	94
IgG1 K_D [nM]	0.18	1	65	0.21	3.07	0.44	0.67	0.68
IgG3 K_D [nM]	NA	0.23	0.76	0.26	2.03	0.17	0.11	0.19
Native subclass	IgG3	IgG1	IgG1	NA	IgG1	IgG1	IgG1	NA





F

Antibody	11	36	57	59	66	77	81	94
IgG1 K_D(nM)	0.1	1.2	5.2	0.23	0.38	0.16	0.20	16
IgG3 K_D(nM)	2.6	1	2.2	0.21	0.47	0.15	0.14	19
IgG1 k_A ($M^{-1}S^{-1}$)	2.56E+05	1.18E+05	1.74E+05	1.30E+06	9.46E+05	1.58E+06	1.74E+06	7.83E+04
IgG3 k_A ($M^{-1}S^{-1}$)	4.36E+05	1.45E+05	5.06E+05	1.56E+06	1.02E+06	1.67E+06	1.95E+06	4.37E+04
Analyte	Spike	Spike	Spike	RBD	RBD	RBD	RBD	NTD

Switching the IgG1 constant domain to IgG3 can alter the avidity for Spike protein. (*A*) Schematic of the heavy and light chain plasmids containing the variable and constant domains. The generation of IgG3 mAbs entails switching the constant domain of the heavy chain from IgG1 (blue) to IgG3 (orange). (*B*) Spike-coated microspheres are used as a model for SARS-CoV-2 virions. Antibody binding assay is done by opsonizing Spike beads with mAbs and adding a secondary Alexa 488-conjugated antibody that reports IgG binding to Spike beads. (*C*) Binding curves showing the percentage of IgG positive Spike beads as a function of IgG concentration. Each clone is shown with both subclasses present (IgG1 in blue and IgG3 in red). Three independent experiments were performed with the mean value shown in the graph and error bars representing the SEM. (*D*) Table summarizing the subclass K_D-values and original subclass for each clone. Avidity was calculated by using a nonlinear regression model in GraphPad Prism. (*E*) Surface plasmon resonance-based binding kinetics with whole spike protein (trimer). Binding of 40 nM Spike-protein (green) to immobilized IgG compared to 40nM RBD (blue) and PBS (pink) for clones 11, 36, and 57 and their respective subclasses. *F*) Table with KD-values and KA for the different subclasses across all clones (11 to 94) and what analyte (spike, RBD, or NTD) was used to measure it with the SPR-based assay. (*G*) ELISA data with spike-coated wells and bound IgG is shown for clones 11 and 57 and their respective subclasses. Three independent experiments were performed with titration curves to plot binding curves. Here, one representative experiment with 4 technical repeats is shown with mean value and error bars representing the SEM. Statistical analysis was performed using a two-tailed *t* test. *P* value above 0.05 denotes ns, *P* value below 0.05 denote *, *P* value below 0.01 denotes **, *P* value below 0.001 denotes ***, and *P* value below 0.0001 denotes ****. Credit: *Proceedings of the National Academy of Sciences* (2023). DOI: 10.1073/pnas.2217590120

Is it possible to improve the antibodies that the body produces to fight SARS-CoV2? In a study led by researchers from Lund University in Sweden, this was investigated by redesigning antibodies and combining them against the virus. The modified antibodies have been tested in human cells and with mice.

Many antibodies used to treat COVID infection during the pandemic have been so-called neutralizing antibodies that prevent the virus from infecting the cell. But as the virus has mutated, the ability of these antibodies to bind to the virus has been lost, and thus also their protective effect. In this study, the researchers focused instead on antibodies that can tag the virus to be eliminated by the immune systems patrolling immune cells, a process called opsonization (see fact box).

"There is often talk about wanting to neutralize viruses by preventing them from binding to the body's cells. It can work well, but we also want to trigger the immune system's ability to remove the virus, which can be done through opsonizing antibodies that mark the virus so it can be eliminated," explains Pontus Nordenfelt, associate professor and researcher in infectious medicine who led the study, published in *PNAS*.

Monoclonal antibodies come from a single clone and are grown in the laboratory in cells for treatment or diagnostics for various diseases. In the current study, the researchers have modified eight such opsonizing monoclonal antibodies by replacing the parts that signal the immune system to respond. Then it was investigated whether different combinations of the antibodies could improve their function. When the researchers switched the backbone of the Y-shaped antibody of one of the most common IgG antibodies in the blood, IgG1, to the backbone of a theoretically more potent antibody, IgG3, they saw a much stronger immune response. The studies were carried out in human cells and with mice.

"Our preclinical results with human immune cells from donors suggest that a cocktail of these IgG3 antibodies could have a potent clinical effect against SARS-CoV-2 and its variants where vaccines do not provide optimal protection," says Arman Izadi, first author of the study and Ph.D. student in Pontus Nordenfelt's research group and doctor (MD) at Skåne University Hospital in Lund.

The monoclonal antibodies the researchers designed can also bind to several sites on the same spike protein. This improves the possibility of protection, say the researchers:

"The strong effect we see with our cocktails is probably explained by the fact that there are more antibodies in different places of the spike protein that 'wave' to immune cells and show where the virus is. Interestingly, this effect was greatest and most pronounced with IgG3 cocktails and not with a cocktail of the original IgG1s. This speaks even more to the fact that IgG3-modified antibodies are promising for treatment," says Arman Izadi.

The researchers have access to many antibodies against SARS-CoV-2, of which eight are of the IgG3 type. The next step in the research is to investigate whether these bind to and protect against the latest virus variants.

"This way of designing the antibodies to enhance their signaling ability opens new ways to treat SARS-CoV-2 infections. We already have promising data, and should this work as we think, an antibody can be developed to protect against all variants of SARS-CoV-2. Even future variants of the virus," says Pontus Nordenfelt.

More information: Arman Izadi et al, Subclass-switched anti-spike IgG3 oligoclonal cocktails strongly enhance Fc-mediated opsonization, *Proceedings of the National Academy of Sciences* (2023). DOI: 10.1073/pnas.2217590120

Provided by Lund University