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Impact of FcRn antagonism on vaccine-induced protective immune responses against viral challenge in COVID-19 and influenza mouse vaccination models

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ABSTRACT

Antagonism of the neonatal Fc receptor through an engineered antibody Fc fragment, such as efgartigimod, results in a decrease in immunoglobulin G levels. This approach is being evaluated as a therapeutic strategy for the treatment of IgG-mediated autoimmune diseases. Our goal was to evaluate the impact of mFc-ABDEG, a mouse-adapted antibody Fc fragment with a mode of action highly similar to efgartigimod, on vaccine-induced protective immune responses against viral infections. Therefore, mouse vaccination models for COVID-19 and influenza were employed, utilizing an mRNA COVID-19 vaccine (COMIRNATY) and an adjuvanted, inactivated quadrivalent influenza vaccine (Seqirus+AddaVax), respectively. In both models, vaccination induced robust humoral responses. As expected, animals treated with mFc-ABDEG had lower levels of virus-specific IgG, while virus-specific IgM responses remained unaffected. The COVID-19 vaccine induced a strong Th1-type T cell response irrespective of mFc-ABDEG treatment. Influenza vaccination resulted in a poor T cell induction, regardless of mFc-ABDEG treatment, due to the Th2-biased response that inactivated influenza vaccines typically induce. Importantly, mFc-ABDEG treatment had no effect on protective immunity against live viral challenges in both models. Vaccinated animals treated with mFc-ABDEG were equally protected as the non-treated vaccinated controls. These non-clinical data demonstrate that FcRn antagonism with mFc-ABDEG did not affect the generation of vaccine-induced protective humoral and cellular responses, or protection against viral challenges. These data substantiate the clinical observations that, although IgG titers were reduced, FcRn antagonism with efgartigimod did not impair the ability to generate new specific IgG responses, regardless of the timing of vaccination.

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Introduction

The neonatal Fc receptor (FcRn) is an MHC class I – like molecule that is widely distributed across mammalian organs, tissues and cells. Its crucial role lies in preserving immunoglobulin G (IgG) and albumin levels by preventing their lysosomal degradation. Pathogenic IgG autoantibodies are associated with many autoimmune diseases such as myasthenia gravis (MG). In MG, a rare neuromuscular autoimmune disease, IgG autoantibodies directly attack neuromuscular junction proteins, leading to failure in neuromuscular transmission and causing potentially life-threatening muscle weakness. Blocking the FcRn-mediated IgG recycling emerges as a promising therapeutic approach for the treatment of MG and other IgG-mediated autoimmune diseases.[1]

Efgartigimod is an antibody fragment that targets FcRn. It is a recombinant human IgG1-derived Fc fragment engineered with ABDEG™ mutations (M252Y/S254T/T256E/H433K/N434F) to enhance its affinity for FcRn at both physiological and acidic pH levels while maintaining the pH-dependent characteristics of FcRn interactions (higher affinity at pH 6.0 compared to near-neutral pH). Efgartigimod competes with endogenous IgG to occupy FcRn, thereby preventing IgG recycling and leading to increased lysosomal degradation of IgG.[2] Clinical studies have consistently demonstrated that efgartigimod rapidly reduces total IgG levels, including IgG autoantibodies, without lowering the levels of other immunoglobulins or albumin.[3–6] Efgartigimod has received regulatory approval for the treatment of generalized Myasthenia Gravis and is currently under evaluation for its potential in treating other IgG-driven autoimmune diseases.

Individuals with autoimmune diseases are more prone to infections due to their autoimmune disorder, elevated comorbidity, and the use of immunosuppressive and immunomodulating medications. In the case of vaccine-preventable diseases like COVID-19 or influenza, an effective vaccination is highly recommended to mitigate the risk

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of severe disease outcomes. Nevertheless, the efficacy of vaccines may be impacted by the concurrent administration of immunosuppressive and immunomodulating drugs. It is recommended to adjust the dosing intervals or temporarily suspend the medication in order to preserve vaccine efficacy for patients under treatment with immunosuppressives and/or immunomodulating agents.[7–17] FcRn blockers reduce IgG levels to a nadir without impacting its production. Importantly, the antagonism of FcRn does not influence other components of the innate and adaptive immune systems. The decrease in IgG induced by FcRn blockers is transient and reversible, signifying that B cells and plasma cells remain unaltered.[18]

The safety of immunization with live or live-attenuated vaccines and the response to immunization with these vaccines during treatment with efgartigimod are unknown. For patients that are being treated with efgartigimod, vaccination with live or live-attenuated vaccines is generally not recommended. If vaccination with live or live-attenuated vaccines is required, these vaccines should be administered at least 4 weeks before treatment and at least 2 weeks after the last dose of efgartigimod. Other vaccines may be administered as needed at any time during treatment with efgartigimod.[19] Clinical study data indicated that patients undergoing efgartigimod treatment maintain their capacity to generate an IgG response to vaccinations. Furthermore, the levels of virus-specific antibodies were sustained, exhibiting a correlation with the kinetics of total IgG reduction.[20–23]

Our objective was to generate non-clinical data that could substantiate the clinical observations noted for efgartigimod and provide insight into the underlying mechanisms of vaccine-induced protective immunity when subjected to treatment with an FcRn blocker. Therefore, we used mouse vaccination models for COVID-19 and influenza to assess the effect of mFc-ABDEG, a mouse-adapted antibody Fc fragment with highly similar functionality to that of efgartigimod, on vaccine-induced humoral and cellular responses, as well as protective immunity against a viral challenge. The animals were vaccinated with T cell dependent vaccines, either an mRNA COVID-19 vaccine (COMIRNATY) or an adjuvanted, inactivated quadrivalent influenza vaccine (Seqirus+AddaVax). We analyzed the humoral responses by measuring the levels of antigen-specific binding and neutralizing antibodies, as well as antigen-specific T cell responses. We assessed the immune protection by examining the animals' resistance to a live virus challenge.

Materials and methods

The experimental protocols were directly used or adapted from the methods described by Jangra et al. (2021).[24]

Mice

6–8 weeks old 129S1 and C57BL/6 mice were purchased from Jackson Laboratories for the COVID-19 and influenza model respectively and housed in a specified pathogen-free facility at Icahn school of medicine at Mount Sinai, with food and water *ad libitum*, adhering to the guidelines from the American Association for Laboratory Animal Science (https://www.aalas.org/).

Mouse surrogate of efgartigimod

Mouse IgG2a-Fc and IgG2c-Fc containing ABDEG mutations are mouse-adapted antibody Fc fragments that exhibit a similar mode of action to that of efgartigimod (referred to as "mFc-ABDEG") and were used in the COVID-19 and influenza models, respectively.[25]

COVID-19 vaccine

SARS-CoV-2 mRNA vaccine from Pfizer (COMIRNATY) was obtained for research purposes as residuals that were recuperated from the Mount Sinai Hospital vaccination pod and stored at −80°C until further use. Vaccine has initially been thawed and was kept at 4°C until frozen again. Vaccine was diluted in PBS (sterile Phosphate Buffer Saline). Stability of the vaccine under the described storage conditions had been confirmed in prior experiments.

Influenza vaccine

Quadrivalent inactivated influenza vaccine (QIV) used in this study was the human Seqirus vaccine (2018–2019 formula) containing the antigens of the following influenza virus strains: A/Singapore/GP1908/2015 IVR-180 (H1N1) (an A/Michigan/45/2015-like virus); A/North Carolina/04/2016 (H3N2) (an A/Singapore/INFIMH-16-0019/2016 -like virus); B/Iowa/06/2017 (a B/Colorado/06/2017-like virus); B/Singapore/INFTT-16-0610/2016 (a B/Phuket/3073/2013-like virus). Vaccine was obtained from the Mount Sinai research pharmacy and was mixed with MF59-like adjuvant (AddaVax). MF59-like adjuvant AddaVax was purchased from Invivogen and was mixed at a 3:1 ratio Vaccine:AddaVax per the manufacturer's recommendation.

Viruses and virus challenge

COMIRNATY-vaccinated mice were infected 14 days post-boost vaccination with 1×10^4 PFU of mouse-adapted SARS-CoV-2 per mice. The mouse-adapted SARS-CoV-2 strain was obtained through serial passage of USA-WA1/2020 in mice as described previously.[26] Body weights were recorded to assess the morbidity during the days post-viral challenge. QIV-vaccinated mice were infected 24 days post-vaccination with 100× lethal dose 50 (18,000 PFU) of egg-grown influenza IVR-180 h1N1 virus, a vaccine strain that contains the surface antigens of influenza A/Singapore/gp1908/2015 (H1N1) virus. All virus infections were performed via intranasal instillation of a 50 µL droplet under mild anesthesia (ketamine (100 mg/kg) and xylazine (10 mg/kg) administered in 150 μL total volume in PBS via the intraperitoneal route). Body weights were recorded to assess the morbidity during the days post-challenge.

COVID-19 model used in this study

The COVID-19 mouse model used in the described studies was chosen at the time as a function of availability at the

height of the COVID pandemic and scientific suitability: During the initial months of the COVID-19 pandemic, mouse models were limited to the K18-hACE2 mouse model that was originally developed for SARS-CoV infection studies and access to this mouse strain was very limited due to high demand. These mice express the hACE2 receptor under the K18 keratinocyte promoter, which allows expression outside of the respiratory tract as well, especially in brain cells. As a consequence, brain infection is a common feature in this mouse model, drastically driving pathology, morbidity and mortality in this mouse model, which is not reflective of human disease.[27] To address this and other issues linked to hACE2 expression in tissues other than the respiratory tract, mice which express hACE2 under the original mouse promoter were developed in collaboration with our laboratory.[28] However, we also observed that SARS-CoV-2 infection resulted in more attenuated morbidity in these mice compared to the K18-hACE2 model. Moreover, these mice became available much later than when these studies were performed. The best option seemed to use a virus that could be adapted to infection of wild type mouse strains, provided it acquired the N501Y mutation in the spike receptor binding region.

Mouse study design – administration protocol

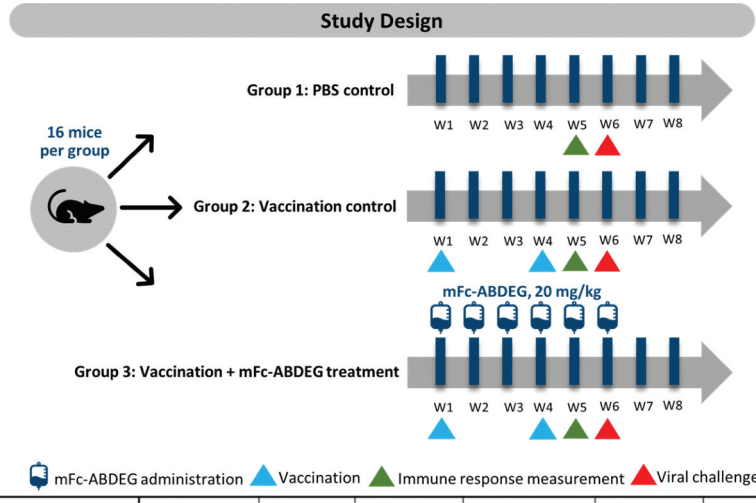
A total of 16 mice (8 female/8 male) were vaccinated with a prime and boost vaccination on respectively day 1 and day 22 with either COMIRNATY or QIV adjuvanted with MF59-like adjuvant (AddaVax) or Phosphate Buffered Saline (PBS) (Figure 1). For SARS-CoV-2 mRNA vaccine (COMIRNATY), equivalent of 3 µg mRNA/mouse were injected in 50 µl volume diluted in PBS via the intramuscular route with a BD U-100 insulin Micro-Fine syringe in the hamstring muscles of the left hind leg. QIV adjuvanted with AddaVax was injected via the intramuscular route with a BD U-100 insulin Micro-Fine syringe in the hamstring muscles of both hind legs (50 µL/leg). The administered vaccine dose corresponded to 1.5 µg of each hemagglutinin type in the vaccine per mouse. PBS was injected via the intramuscular route with a BD U-100 insulin Micro-Fine syringe in the hamstring muscles of the left hind leg for COVID-19 model and in both hind legs for influenza model.

Animals were divided into three study groups: (1) PBS control group (not vaccinated), (2) Vaccinated control group, and (3) Treated group (vaccinated and treated with mFc-ABDEG). Within each group, a subset of four animals (two female/two male) was dedicated to assessing the cellular immune response to vaccination and were sacrificed before the viral challenge (Day 32). The mFc-ABDEG treatment started three days prior to vaccination and continued throughout the immune response development and viral challenge until day 37. mFc-ABDEG was administered subcutaneously in the neck twice per week at a dosage of 20 mg/kg.

Serology

Anti-SARS-CoV-2 spike and anti-HA IgG and IgM ELISA

Maxisorp Nunc 96-well microtiter plates were coated with stabilized soluble trimeric COVID-19 spike protein



	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
mFc-ABDEG administration	Day -3, 2, 5	Day 9, 12	Day 16, 19	Day 23, 26	Day 30, 33	Day 37		
Vaccination	Prime Day 1			Boost Day 22				
Viral challenge						Day 36		
Humoral response measurement					Day 30			
Cellular response measurement					Day 32			
Total IgG measurement	Day -3, 2	Day 9	Day 16	Day 23	Day 30	Day 37	Day 44	Day 50
Splenocytes isolation					Day 32			
Lung harvest COVID-19						Day 39		
Lung harvest Influenza						Day 40		
Serum sampling	Day -3, 2	Day 9	Day 16	Day 23	Day 30	Day 37	Day 44	Day 50
Weight measurement						Day 36 - 50 (daily)		

Figure 1. Experimental design for mouse immunization and mFc-ABDEG-treatment in the COVID-19 and influenza model.

(2 µg/ml) and QIV (2 µg/ml HA equivalent for each HA) in bicarbonate buffer and incubated overnight at 4°C. After washing and blocking with 5% milk for 1 h at room temperature, serum samples were 3-fold diluted starting at 1/100 dilution in PBS with 0.05% Tween20 and were allowed to bind ELISA antigen for 1.5 h at room temperature. Plates were washed three times with PBS (0.05% Tween20) and incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies targeting mouse IgG (GE Healthcare). After a final washing step, tetramethylbenzidine (TMB) substrate (Sigma-Aldrich) is used to estimate levels of spike-specific or HA-specific mouse IgG by measuring the OD_{450} with the OD_{650} as a reference after stopping the colorimetric reaction with 1 M H_2SO_4. A modification of the assay to detect spike-specific and HA-specific IgM was performed by following the above-described protocol but by incubating with IgM-specific HRP-conjugated secondary antibodies instead of total IgG-specific secondary antibodies.

SARS-CoV-2 microneutralization assay

In brief, mRNA-vaccinated sera were inactivated at 56°C for 30 min. Serum samples were serially diluted 2-fold starting from a 1:10 dilution in infection medium (DMEM +2% FBS + 1X non-essential amino acids). The samples were then incubated with 100× tissue culture infective dose 50 (TCID50) which equals 40 plaque forming units (PFU) of mouse-adapted SARS-CoV2 virus for 1 hour in an incubator at 37°C, 5% CO2 and subsequently transferred onto pre-seeded Vero-E6 cells in 96-well cell-culture plates. The plates were incubated at 37°C for 48 hours and fixed in 4% formaldehyde. The cells were washed with 1XPBS and blocked in 5% milk in 1X PBS + 0.1% Tween20 for 1 hour at room temperature. After blocking, the cells were permeabilized with 0.1% TritonX100, washed and incubated with anti-SARS-CoV-2-nucleoprotein and anti-SARS-CoV-2-spike monoclonal antibodies, mixed in 1:1 ratio, for 1.5 hours at room temperature. The cells were washed again and incubated with HRP-conjugated anti-mouse IgG secondary antibody for 1 hour at room temperature followed by a brief PBS wash. Finally, 50 µl tetramethyl benzidine (TMB) substrate was added and incubated until blue color appeared and the reaction was terminated with 50 µL 1 M H_2SO_4. The absorbance at 450 nm (OD_{450}) was recorded and percentage inhibition calculated.

Hemagglutination inhibition assay (H1N1 virus neutralizing antibodies)

Four volumes of receptor destroying enzyme (RDE, Vibrio cholera filtrate, Sigma Aldrich) were added to each volume of mouse serum. After overnight incubation at 37°C, sera were heat inactivated at 56°C for 30 min in sodium citrate buffer. Four hemagglutination units of IVR-180 h1N1 virus were mixed with twofold serial dilutions of treated sera in a final volume of 50 µL. Mixtures of virus and diluted serum were allowed to bind for 1 h at room temperature before 50 µL of 0.5% chicken red blood cell suspension is added. HI titers were read after 1 h incubation on ice.

Lung virus titers (plaque assay)

Whole lungs were harvested from the mice and homogenized in 0.5 mL 1XPBS. After brief centrifugation, the tissue debris was discarded, and the supernatant is 10-fold serially diluted starting from 1:10 dilution.

For assessments of IVR-180 h1N1 virus titers, MDCK cells were incubated with the lung homogenate dilutions for 1 hour at 37°C, 5% CO2 and then overlaid with a mixture of 2% oxoid agar and 2X minimal essential medium (MEM) supplemented with 1% diethyl-aminoethyl (DEAE)-dextran and 1 µg/mL L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin. After 48 hours of incubation at 37°C, 5% CO2, the plates were fixed in 4% formaldehyde and immune-stained with IVR-180 post-challenge polyclonal serum.

For assessments of SARS-CoV-2 titers, pre-seeded Vero-E6 cells were incubated with diluted lung homogenates for 1 hour at room temperature and then overlaid with a 1 mL mixture of 2% oxoid agar and 2X MEM supplemented with 2% FBS. After 72 hours of incubation at 37°C with 5% CO2, the plates were fixed in 4% formaldehyde, permeabilized with 0.1% Triton-X100 followed by immune-staining of infected cells with anti-mouse SARS-CoV-2 nucleoprotein and anti-mouse SARS-CoV -2 spike monoclonal antibodies. After incubation with primary antibodies, HRP-conjugated anti-mouse secondary antibody was added for 1 hour. Finally, the plaques were developed with TrueBlue substrate (KPL Seracare). The final viral titers were calculated in terms of plaque forming units (PFU)/mL.

IFNγ ELISpot assay

IFN-γ enzyme-linked immunospot assay kits were purchased from R&D Systems (cat. No. EL485). The assay was performed according to the manufacturer's protocol. Briefly, sterile monoclonal anti-IFN-γ antibody-coated 96-well immunoplates were blocked with cell culture media (RPMI-1640 supplemented with 10% FBS, 100 IU Pen-Strep, and 1X GlutaMAX). On day 10 after vaccination, the spleens of vaccinated mice were isolated aseptically and splenocytes single cell suspensions were prepared by forcing spleens through a 70 µm cell strainer into cell culture media. After lysis of RBCs with NH_4Cl solution, 1×10^5 splenocytes were plated in 100 µl of culture medium supplemented with the restimulation peptide pool at a final concentration of 0.6 nmol/mL. High-pressure liquid chromatography – purified peptides (>95% purity) were purchased from Miltenyi (cat. No. 130-099-803 and 130-127-953). Phorbol myristate acetate (PMA)/ionomycin was used as positive control (ThermoFisher, cat. no. 00-4970-03). After 16 h of peptide restimulation at 37°C, plates were washed with the wash buffer in the kit and IFN-γ trapped on the plates was detected by a biotinylated polyclonal anti-IFN-γ followed by enzyme-linked streptavidin-alkaline phosphatase conjugate. After BCIP (5-Bromo-4-chloro-3-indolyl phosphate)/NBT (nitro blue tetrazolium) substrate addition, spots occurred at places where immune cells secreted IFN-γ during peptide-restimulation. The spots were counted using an inverted light microscope.

Intracellular Cytokine Staining (ICS)

On day 10 after vaccination, the spleens of vaccinated mice were isolated aseptically and splenocytes were prepared.

After lysis of RBCs with NH_4Cl solution, 2×10^6 splenocytes were plated in 200 µL of culture medium (RPMI-1640 supplemented with 10% FBS, 100 IU Pen-Strep, and 1X GlutaMAX) with the restimulation peptide pool at a final concentration of 0.6 nmol/mL. High-pressure liquid chromatography – purified peptides (>95% purity) were purchased from Miltenyi (cat. No. 130-099-803 and 130-127-953). Phorbol myristate acetate (PMA)/ionomycin was used as positive control (ThermoFisher, cat. no. 00-4970-03). After 6 h of peptide restimulation in the presence of protein transport inhibitors (ThermoFisher, cat. no. 00-4980-03), cells were collected, washed, stained for surface markers (CD3 Alexa Fluor 700, CD8 PerCP, CD4 BV510, CD44 PE-CF594) and viability (Fixable Viability Dye eFluor 520). Subsequently cells were fixed with Fix/Perm buffer (BD), permeabilized with 1X Perm/Wash buffer (BD) and stained for intracellular IFN-γ (Alexa Fluor 647) and IL-4 (PE-Cy7). The amount of live IFN-γ+ CD4 + and CD8+ T cells was quantified using Beckman Coulter Gallios flow cytometer.

Multiplex cytokine ELISA

Splenocytes were prepared and RBC were lysed as described above. 2×10^6 splenocytes were plated in 200 µL of culture medium (RPMI-1640 supplemented with 10% FBS, 100 IU Pen/Strep, 1X GlutaMAX, 1% non-essential amino acids, 1 mm sodium pyruvate, and 10 mm HEPES) supplemented with restimulation peptide pool at a final concentration of 0.6 nmol/mL in round bottom plates for 48 h. Phorbol myristate acetate (PMA)/ionomycin was used as positive control (ThermoFisher, cat. no. 00-4970-03). After stimulation, cells were centrifuged at 400 × *g* for 5 minutes at 4°C and supernatants were collected and stored at −20°C. Levels of Th1/Th2 associated cytokines were measured in 150 µL of the culture supernatant using a bead-based Luminex assay (cat. no. EPX110 -20,820-901) according to the manufacturer's recommendations on a Millipore-Luminex L X 200reader.

Histology

Mice were subjected to terminal anesthesia and euthanasia (5× anesthesia dose) and left lung lobes were inflated with 10% formaldehyde. Fixed lungs were sent to the Mount Sinai Pathology Care Facility for paraffin embedding, tissue analysis, and scoring by an independent veterinary pathologist who was blinded to the study groups. Hematoxylin and eosin (H&E) staining was performed. Scores were assigned by the pathologist based on multiple parameters: Percentage of lung area affected (1= ≤25%, 2 = 25–50%, 3 = 50–75%, 4 = 100%), peri bronchial inflammation, epithelial degeneration/necrosis of bronchi/bronchioles, bronchial/bronchiolar inflammation, intraluminal cell debris in bronchi/bronchioles, alveolar inflammation, alveolar epithelial cells necrosis, fibrin deposition and hyperplasia of type II pneumocytes. A 5-point scoring system was used ranging from 0–4, with 0 indicating no epithelial degeneration/necrosis and inflammation while 4 indicating severe epithelial degeneration/necrosis and inflammation (1=mild, 2=moderate, 3=marked, 4=severe).

Total IgG levels

MaxiSorp Nunc 96-well high protein-binding capacity plates were coated with 100 µL per well of goat anti-mouse IgG fab antibody (Tebu-bio #MAB12775) at 1 µg/mL in PBS and incubated overnight at 4°C. The plates were then washed three times with 1X PBS + 0.05% Tween20 and blocked with 1% Casein -PBS for two hours at room temperature while shaking at 450 rpm. Samples were diluted 10.000-times in 0.1% Casein-PBS. A standard dilution series of 11 points (2-times dilutions) starting from 1000 ng/mL were prepared in 0.1% Casein-PBS (reference standard: Tebu-bio #IMSIGGAP10MG). High, medium, and low QC samples, at a concentration of 240, 75, and 5 ng/mL respectively, were prepared in 0.1% Casein-PBS. The plates were washed three times with 1X PBS + 0.05% Tween20. Samples, standard dilutions, and QC samples were added to the plates and incubated for 2 hours at room temperature while shaking at 450 rpm. The plates were washed five times with 1X PBS + 0.05% Tween20. Horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG-Fc F(ab')2 (Abcam, #98741), diluted 40.000-times in 0.1% Casein-PBS, was added to the plates and incubated for one hour at RT while shaking at 450 rpm. The plates were washed five times and developed with 100 µL per well of soluble high sensitivity tetramethylbenzidine substrate (s(HS)TMB) (SDT-Reagents for life #sTMB) for 15 minutes. The reaction was terminated with 100 µL 0.5 M H2SO4 and the absorbance was measured at 450 nm with 650 nm as a reference.

Statistical analysis

Titers of specific IgG and IgM, and neutralizing antibodies

The log-transformed endpoint Titer/ID50 values were subjected to a two-way ANOVA model, considering group, sex and their corresponding two-way interaction. Note that a parametric ANOVA model could still be applied since the log-transformation stabilized the variances and improved the normality assumption of the residuals. If the interaction between group and sex proved to be statistically significant, sex-specific comparisons were conducted between the PBS control group (not vaccinated) and the vaccinated group in a first step and between the vaccinated group and the vaccinated mFc-ABDEG treated group in a second step. On the other hand, if the interaction between group and sex was not found to be significant, the comparisons in both steps were pooled across both males and females. A multiplicity adjustment for each step was applied based on a multivariate t-distribution with the same covariance structure as the estimates.

Histology

The statistical methodology described in the preceding section was extended to analyze the histopathology data. An arcsinh-transformation was applied to account for the fact that the data was not strictly positive.

IFNγ ELISpot assay, ICS and multiplex cytokine ELISA

A two-way ANOVA model was used, considering group, and stimulation as independent factors, along with their respective interactions. Both un-transformed and arcsinh-transformed outcomes were examined as well as varied variance weights

for different stimulations were considered. Model assumptions were checked and the model with the most favorable residual diagnostic graphs was chosen for further analysis. Contrasts of interest were calculated in a two-step procedure.

Step 1 compared restimulated cells with SARS-CoV-2 spike (S) or H1N1 peptide pool against unstimulated cells and Vaccinated group. Step 2 compared Vaccine versus Vaccine + mFc-ABDEG and stimulation. Results from Step 2 were only valid if both Step 1 comparisons were significant. Caution was advised in interpreting the results considering the limited number of animals per group ($n = 4 = 2f + 2m$). All hypothesis tests were evaluated at a 5% significance level.

Results

mFc-ABDEG treatment does not affect the generation of a protective humoral COVID-19 vaccine response in the described model

Vaccine-induced humoral responses were analyzed by measuring the levels of antigen-specific binding and neutralizing antibodies. On study day 30 (8 days post-boost vaccination), vaccinated mice mounted spike-specific IgG and IgM immune responses against SARS-CoV-2 spike protein, whereas unvaccinated PBS control mice showed no, or only background response levels (Figure 2a,b). The levels of spike-specific antibodies were statistically significant compared to PBS controls, except for the IgM levels in females, which showed slightly higher background levels measured for the PBS control females. In vaccinated mFc-ABDEG treated mice, a reduction in anti-spike IgG titers were observed in both males and females, as expected based on the pharmacological properties of mFc-ABDEG. However, this effect was not statistically significant ($p = .054$) compared to the vaccinated mice. There was no significant difference in anti-spike IgM titers between vaccinated mFc-ABDEG treated mice and untreated mice.

Vaccination had induced virus-neutralizing antibodies in all vaccinated male and female mice as compared to unvaccinated mice (Figure 2c). The difference in levels of neutralizing antibodies were statistically significant compared to PBS controls ($p < .001$), and in female vaccinated groups, they were significantly higher compared to male vaccinated groups ($p = .026$). It has been described that antibody responses to vaccines are often higher in females than males.[29] Neutralizing antibodies titers were statistically lower ($p < .001$) in mFc-

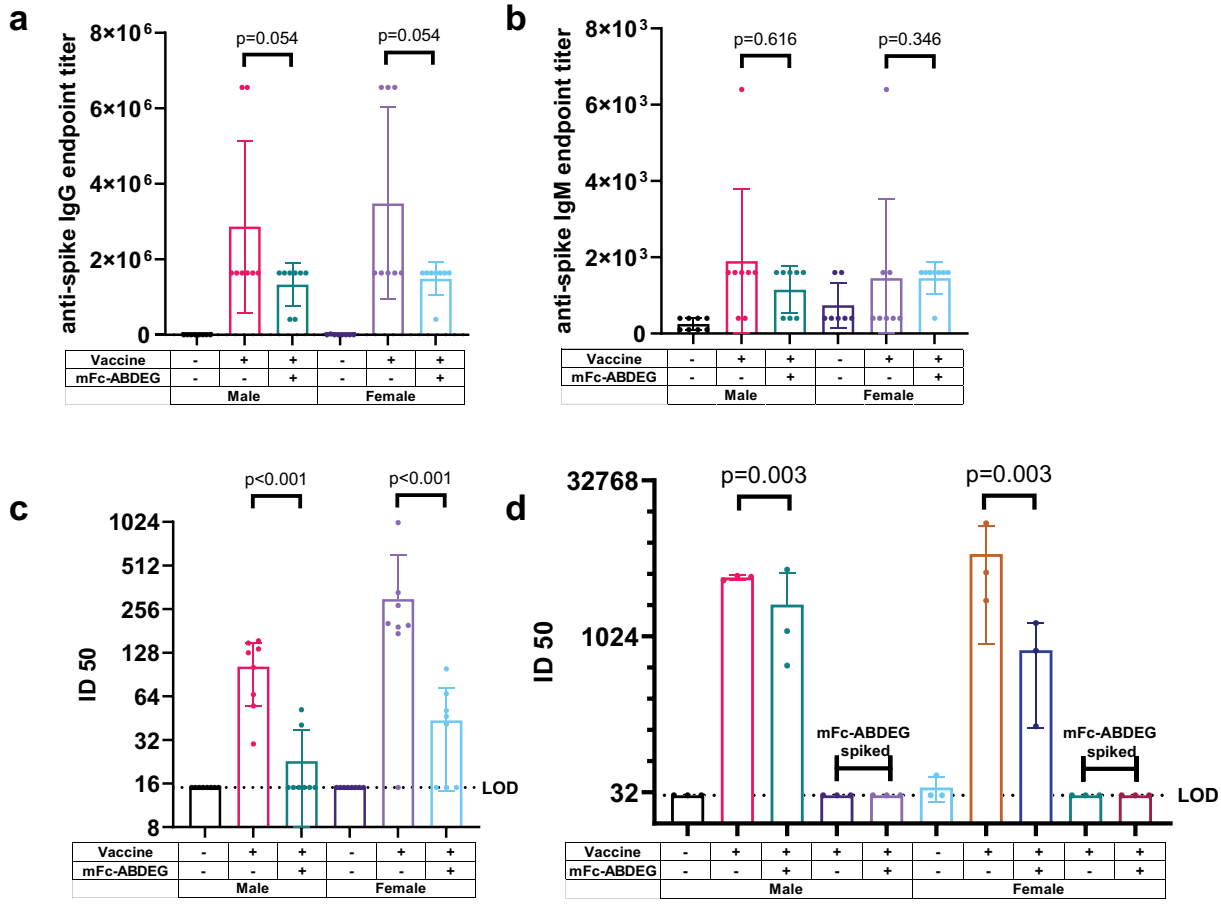


Figure 2. Antibody responses to vaccination in mFc-abdeg-treated animals (COVID-19 model): ELISA was performed on post-boost serum (day 8 post-boost vaccination) to assess the production anti-spike IgG (a) and anti-spike IgM (b) in response to vaccination in mice with or without mFc-ABDEG treatment, along with the non-vaccinated group (16 mice per group – 8 per sex). (c) microneutralization assays were performed on post-boost serum (taken 8 days post-boost vaccination) to measure the virus-neutralizing antibodies using the mouse-adapted SARS-CoV2 virus (16 mice per group – 8 per sex). Note that at this timepoint values presented for mFc-ABDEG treated animals are likely underestimated due to interference of mFc-ABDEG present in the samples (see explanation in text). (d) additional microneutralization assays were conducted on the follow-up samples (taken 28 days post-boost vaccination), to measure the virus-neutralizing antibodies using the mouse-adapted SARS-CoV2 virus (6 mice per group – 3 per sexr). Error bars represent standard deviation.

ABDEG treated animals compared with untreated animals. This reduction in neutralizing antibodies in the mFc-ABDEG treated mice was more pronounced than expected when comparing it to the titers of binding antibodies observed in the same groups. Indeed, some mice in the group that received mFc-ABDEG treatment had antibody titers below the limit of detection (LOD). It has been reported that renal epithelial cells (Vero E6 cell line), used in the neutralizing assay, express FcRn.[30] Consequently, there was a potential for drug interference, as mFc-ABDEG present in the sera may have bound to FcRn on the Vero E6 cells, thereby reducing the levels of neutralizing antibodies during the 48-hour incubation period of the assay.

To further explore the potential drug interference in this assay, titers of neutralizing antibodies were measured in follow-up serum samples taken 14 days after the viral challenge (Day 50). These samples were obtained 13 days after the final administration of mFc-ABDEG, exceeding the estimated wash-out period of mFc-ABDEG in naive mice (with a half-life of approximately 5 hours). In order to further confirm the hypothesis of drug interference, these follow-up samples were reconstituted with mFc-ABDEG. Neutralizing antibodies were detected in all vaccinated groups on study day 50 (28 days post boost vaccination) irrespective of mFc-ABDEG treatment (Figure 2d). Furthermore, the addition of mFc-ABDEG (200 µg/mL) resulted in a complete reduction of neutralizing antibody titers in all vaccinated groups, both with and without mFc-ABDEG treatment, thereby confirming the drug interference in the neutralizing assay.

mFc-ABDEG treatment does not affect the generation of a cellular COVID-19 vaccine response in the described model

Vaccine-induced cellular responses were assessed by measuring the IFN-γ-secreting cells, antigen-experienced (CD44+) IFN-γ+ or IL-4+ CD4 and CD8 T cells and T cell associated cytokines, following stimulation of isolated splenocytes with the Spike (S) peptide pool. On study day 32 (10 days post-boost vaccination), IFN-γ-secreting cells were detected in all vaccinated groups, irrespective of mFc-ABDEG treatment (Figure 3a). The nonspecifically stimulated positive control phorbol myristate acetate (PMA)/ionomycin induced a strong response compared to the unstimulated negative control. Notably, two male samples in the vaccine-only cohort had lower cellular input and were excluded from the ELISPOT quantification.

Antigen experienced (CD44+) IFN-γ+ CD4 and CD8 T cells were activated after S-peptide pool stimulation in both vaccinated groups, with or without mFc-ABDEG treatment (Figure 3b–d). The frequencies of cytokine-secreting, antigen-experienced IFN-γ+ CD4 and CD8 T cells were not significantly affected by mFc-ABDEG treatment. Furthermore, very low frequencies of IL-4+

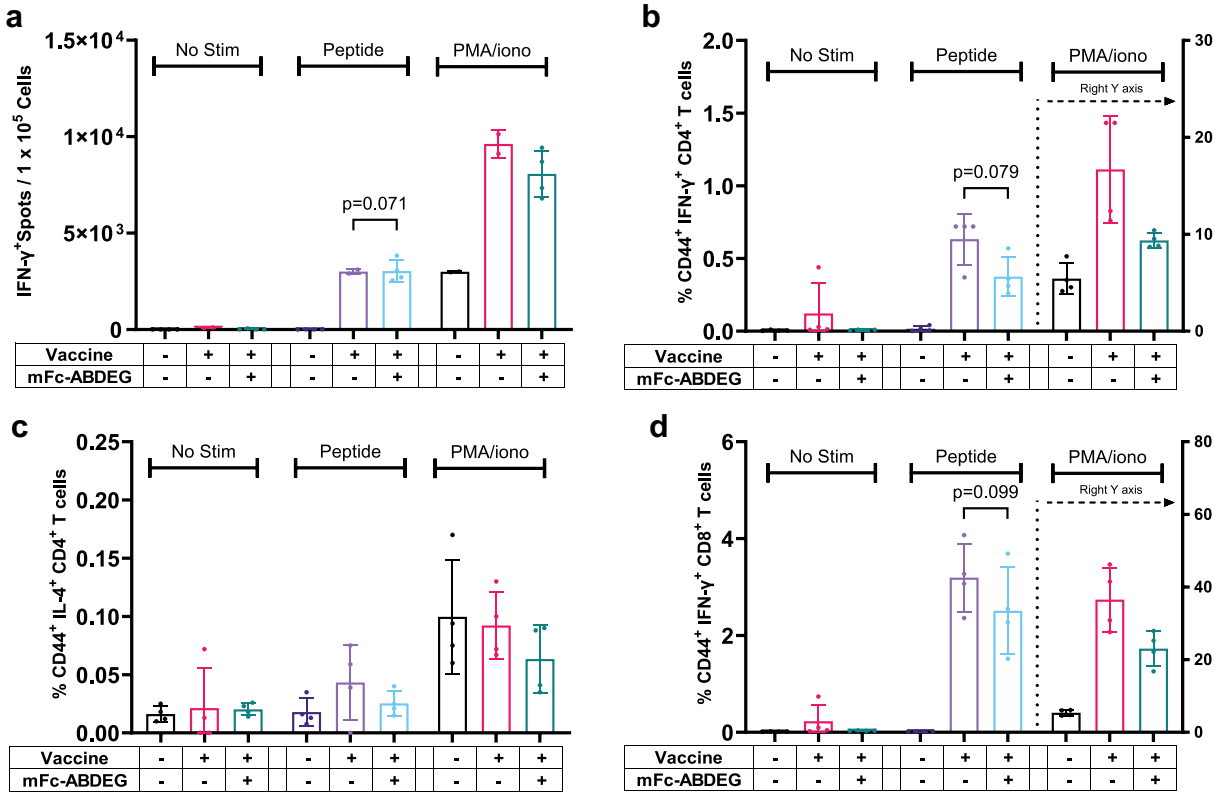


Figure 3. T cell responses to vaccination in mFc-ABDEG-treated animals (COVID-19) (4 mice per group – 2 per sex): (a) IFN-γ-secreting cells in vaccinated mice as measured by ELISPOT. Splenocytes were stimulated with an overlapping S-peptide pool (miltenyi), PMA/ionomycin cocktail, or left unstimulated. Low cell input: less cells than the recommended number of cells was used due to low cell numbers recovered after single cell splenocyte cultures were prepared. (b) S-specific ifn-γ- and IL-4-secreting T cells at 10 days post-vaccination quantified by flow cytometry. T cells (live, singlet, CD3+, CD4+, or CD8+) after 6 h stimulation with a S-peptide pool (miltenyi), PMA/ionomycin, or media (no stimulation) in the presence of protein transport inhibitor. Frequency of antigen-experienced (b) IFN-γ+ CD4 T cells, (c) IL-4+ CD4 T cells, and (d) IFN-γ+ CD8 T cells. Error bars represent standard deviation.

CD4 T cells were reactivated in vaccinated mice, suggesting that vaccination elicited a predominantly Th1-biased immune profile.

In vaccinated mice, T cell associated cytokines (IL-2, IL-4, IFN-γ and TNFα) were secreted following S-peptide pool stimulation, illustrating the T cell responses to vaccination (Figure 4). The stimulation with S-peptide produced a positive but variable response irrespective of mFc-ABDEG treatment. The levels of measured cytokines were similar (IL-2, IFN-γ and TNFα) or significantly higher (IL-4) in mFc-ABDEG treated group compared to non-treated vaccinated control group. Of note, the higher level of IFN-γ response in peptide-stimulated, non-vaccinated group was unexpected and was probably caused by contamination (Figure 4d). In summary, multiple assays used to evaluate T cell responses yielded consistent results, indicating that mFc-ABDEG treatment did not adversely affect vaccine-induced T cell responses.

mFc-ABDEG treatment does not affect vaccine-mediated protection upon SARS-CoV-2 challenge in the described model

Animals were challenged with 10^4 PFU of SARS-CoV-2 virus via the intranasal route. No body weight loss was observed following the virus challenge (Figure 5a). Body weight loss upon experimental infection with the mouse-adapted virus depends on the age of the mice, with transient weight loss particularly observed in older mice (18 weeks).[26] Lungs were collected at 3 days post- infection for virus titration. Vaccination resulted in the absence of detectable virus titers in the lungs of vaccinated mice, and this beneficial effect was not influenced by mFc-ABDEG treatment (Figure 5b). Female mice showed lower virus titers compared to male mice at this time point post infection. Histopathology scoring was performed on lungs collected 3 days post-infection with SARS-CoV-2 virus (Figure 5c, Pathology scoring data can be found in Supplemental material). The observed pathology scores post-infection were generally low, and they were completely absent in vaccinated male mice, irrespective of mFc-ABDEG treatment. In vaccinated female mice, a slight reduction in the average cumulative pathology score was noted when compared with the unvaccinated group, displaying a sex difference in protection from pathology in vaccinated animals. The beneficial effect of vaccination was not impacted by mFc-ABDEG treatment (Figure 5c).

mFc-ABDEG treatment does not affect the generation of a protective humoral influenza vaccine response in the described model

Vaccine-induced humoral responses were analyzed by measuring the levels of antigen-specific binding and neutralizing antibodies. On study day 30 (8 days post-boost vaccination), QIV-vaccinated mice mounted specific IgG and IgM immune responses against influenza HA compared with unvaccinated PBS control mice (Figure 6a,b).

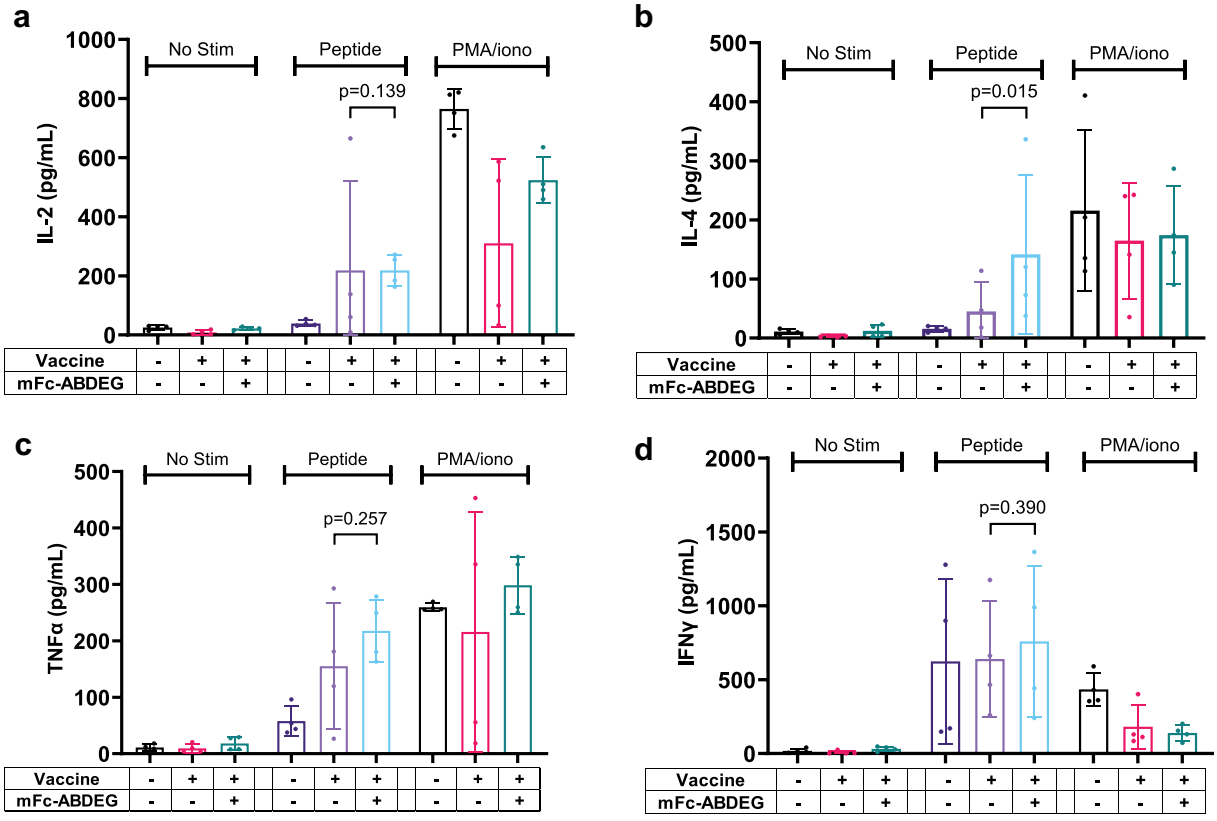


Figure 4. Cytokine levels in culture supernatant of restimulated splenocytes from mFc-ABDEG-treated vaccinated animals (COVID-19) (4 mice per group – 2 per sex): splenocytes were stimulated with an overlapping S-peptide pool (miltenyi), or PMA/ionomycin cocktail, or left unstimulated. Levels of T-cell associated cytokines IL-2 (a), IL-4 (b), IFN-γ (c) and TNFα (d) were measured in culture supernatant by multiplex cytokine ELISA. Error bars represent standard deviation.

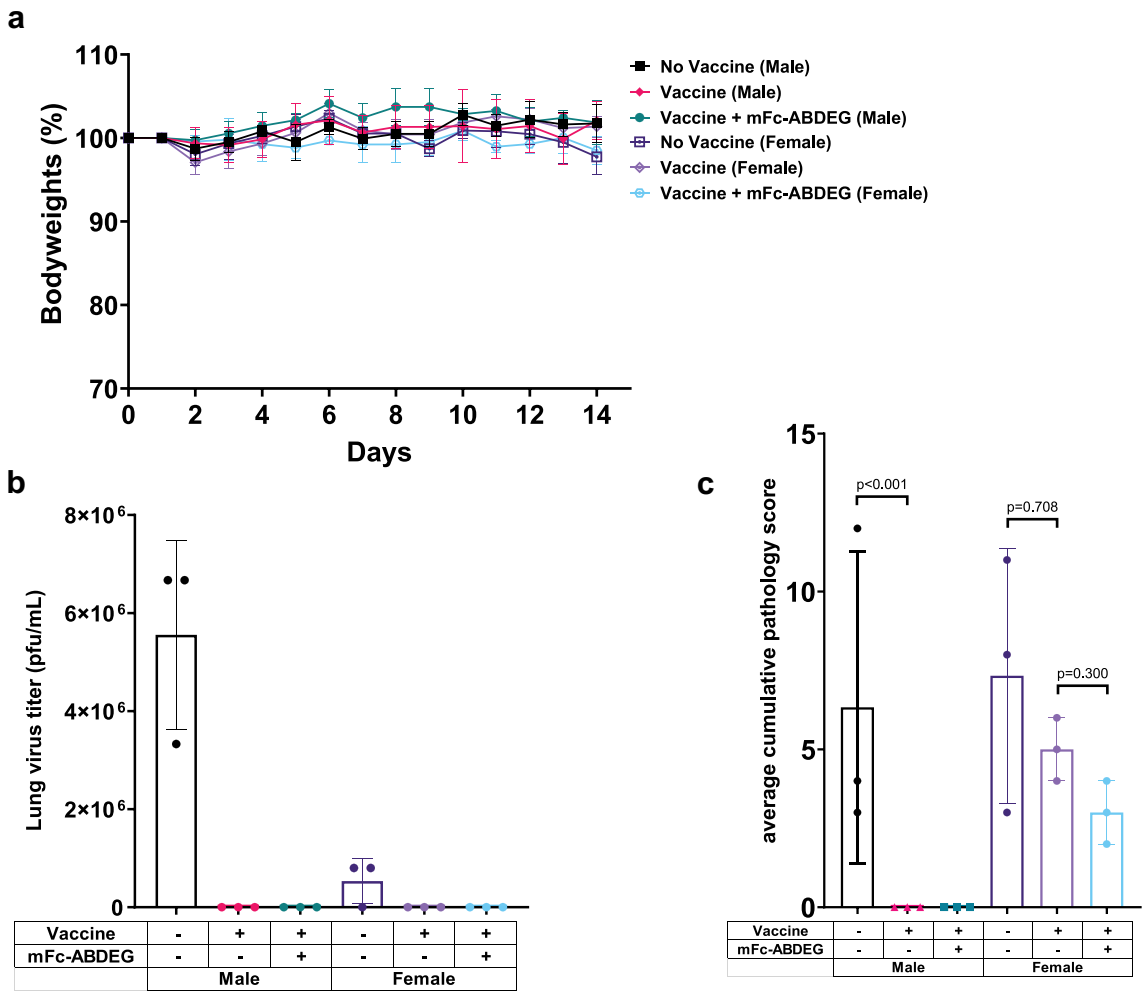


Figure 5. Vaccine-induced immunity protects mice against SARS-CoV-2 infection irrespective of mFc-ABDEG treatment: (a) bodyweight changes in 129S1 mice challenged with mouse-adapted SARS-CoV-2 virus (10,000 PFU) over a period of 14 days (12 mice per group – 6 per sex). (b) lung virus titers in male and female mice at 3 days post infection (6 mice per group – 3 per sex). (c) cumulative pathology score in lungs collected at 3 days post-infection (6 mice per group – 3 per sex). Error bars represent standard deviation.

The levels of H1N1-specific antibodies were statistically significant compared to PBS controls ($p < .001$). In mice treated with mFc-ABDEG, significantly lower ($p < .001$) titers of anti-HA IgG were observed in both males and females, consistent with the pharmacological effect of mFc-ABDEG. No significant difference in anti-HA IgM titers was noted in mFc-ABDEG treated mice compared to untreated vaccinated mice.

QIV vaccination induced hemagglutination inhibition titers reflecting H1N1 virus-neutralizing antibodies in all vaccinated males and female mice compared to unvaccinated mice (Figure 6c).

The difference in levels of neutralizing antibodies were statistically significant compared to PBS controls ($p < .001$), and in female vaccinated group, they were significantly higher compared to male vaccinated group. Titers of neutralizing antibodies were lower in mFc-ABDEG treated female animals compared with untreated female mice ($p < .001$). However, for male mice, the antibody-lowering effect of mFc-ABDEG treatment did not result in a statistically significant difference between vaccinated-treated and -untreated male mice.

mFc-ABDEG treatment does not affect the generation of a cellular influenza vaccine response in the described model

Vaccine-induced cellular responses were evaluated by measuring the IFN-γ-secreting cells, antigen experienced (CD44+) IFN-γ+ or IL-4+ CD4 and CD8 T cells and T cell associated cytokines, after stimulation of isolated splenocytes with the H1N1 peptide pool. On study day 32, a very low number of IFN-γ-secreting cells were observed in response to the QIV vaccination in all vaccinated mice, while a stronger response to positive control (PMA+ionomycin) was noted regardless of mFc-ABDEG treatment (Figure 7a). It is worth mentioning that QIV and other inactivated influenza vaccines have been shown to be poor inducers of T-cell immunity as described previously by Jangra *et al.*[31] Of note, one male sample in the vaccine-only cohort had a lower cellular input and was excluded from the ELISpot quantification.

Frequencies of antigen experienced (CD44+) IFN-γ+ CD4 and CD8 T cells, after stimulation with H1N1 peptide pool, were also notably low in the vaccinated groups with or without mFc-ABDEG treatment (Figure 7b–d). The

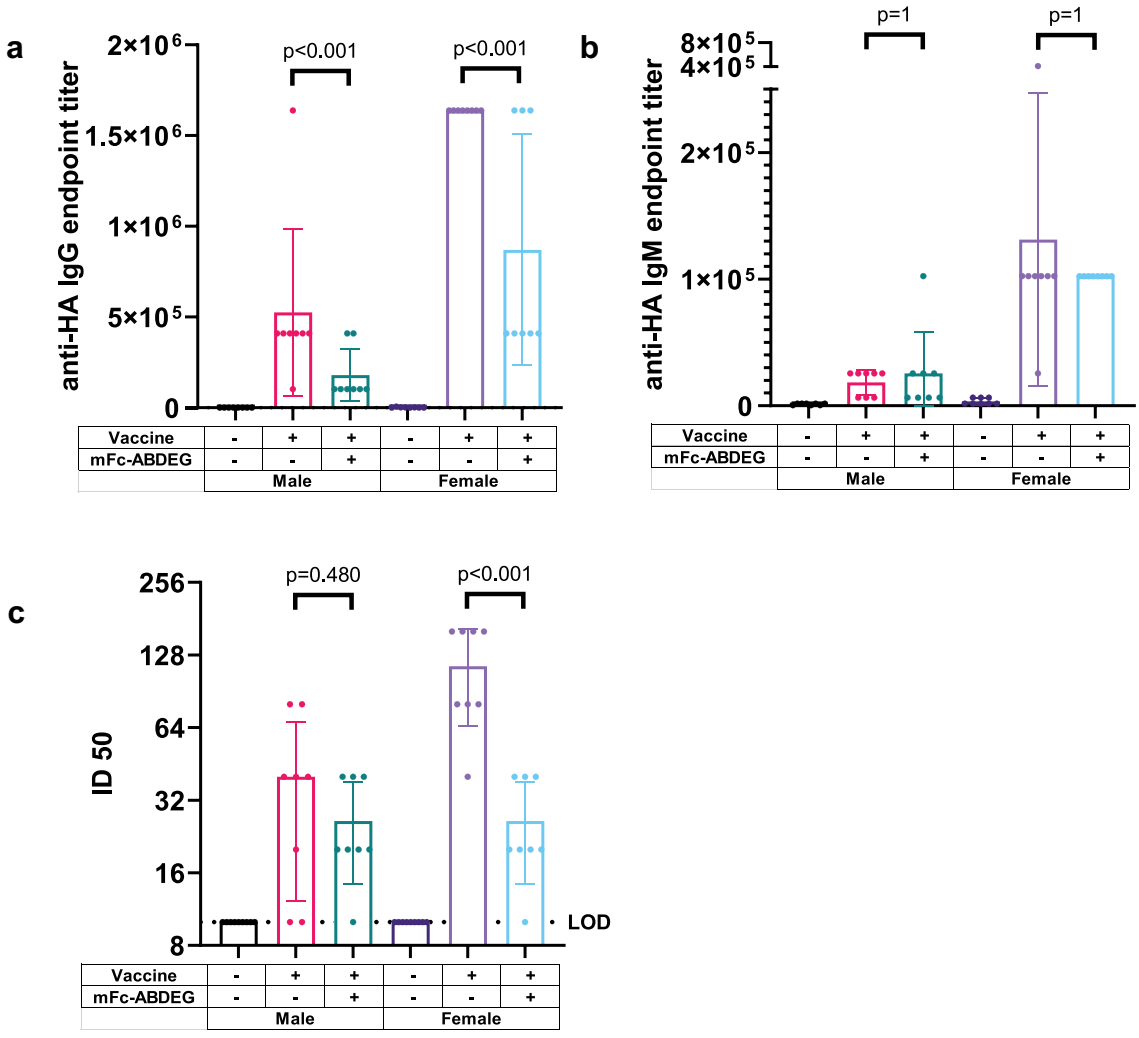


Figure 6. Antibody responses to vaccination in mFc-ABDEG-treated animals (influenza model) (16 mice per group – 8 per sex): ELISA was performed on post-boost serum (day 8 post-boost vaccination) to assess the production of anti-ha IgG (a) and anti-ha IgM (b) in response to vaccination in mice with or without mFc-ABDEG treatment, along with the non-vaccinated group. (c) hemagglutination inhibition (HI) assays were performed on post-boost serum (taken 8 days post-boost vaccination) to measure the virus-neutralizing antibodies using the influenza IVR-180 h1N1 virus. Error bars represent standard deviation.

positive control (PMA+ionomycin) elicited a clear response compared to the unstimulated negative control, irrespective of the mFc-ABDEG treatment. Similarly, the cytokine profile aligns with the minimal T cell response observed in the IFN-γ ELISpot and ICS experiments. In vaccinated groups, the secretion of T cell associated cytokines (IL-2, IL-4, IFN-γ and TNFα) was very weak after H1N1 peptide pool stimulation, indicating a markedly low T cell response to vaccination with QIV (Figure 8). However, these cytokines were induced by the positive control (PMA+ionomycin) regardless of the mFc-ABDEG treatment. Overall, the T cell responses triggered by the positive control in these assays, were not negatively impacted by mFc-ABDEG treatment.

mFc-ABDEG treatment does not affect vaccine-mediated protection upon IVR-180 H1N1 challenge in the described model

Mice that were vaccinated, with or without mFc-ABDEG treatment and subsequently challenged with IVR-180 h1N1 virus, showed no differences in body weight (Figure 9a). In contrast, unvaccinated mice experienced a loss of more than 20% of their initial body weight following viral challenge. All unvaccinated mice were euthanized for humane reasons when their body weight loss reached more than 20% of their initial body weight. Vaccination resulted in complete control of lung virus titers, irrespective of mFc-ABDEG treatment, while unvaccinated mice had detectable lung virus titers (Figure 9b).

mFc-ABDEG treatment results in reduced total IgG levels

Total IgG concentration was measured in all study groups for both COVID-19 and influenza models (Figure 10). In the vaccinated mice without mFc-ABDEG treatment, a clear increase of IgG was observed following both the prime and boost vaccinations, with the latter resulting in a more substantial IgG elevation. Total IgG levels were reduced in the vaccinated mice treated with mFc-ABDEG, as expected, based on its pharmacological properties. On the first day post-viral challenge (*), the reduction in IgG was approximately 80%

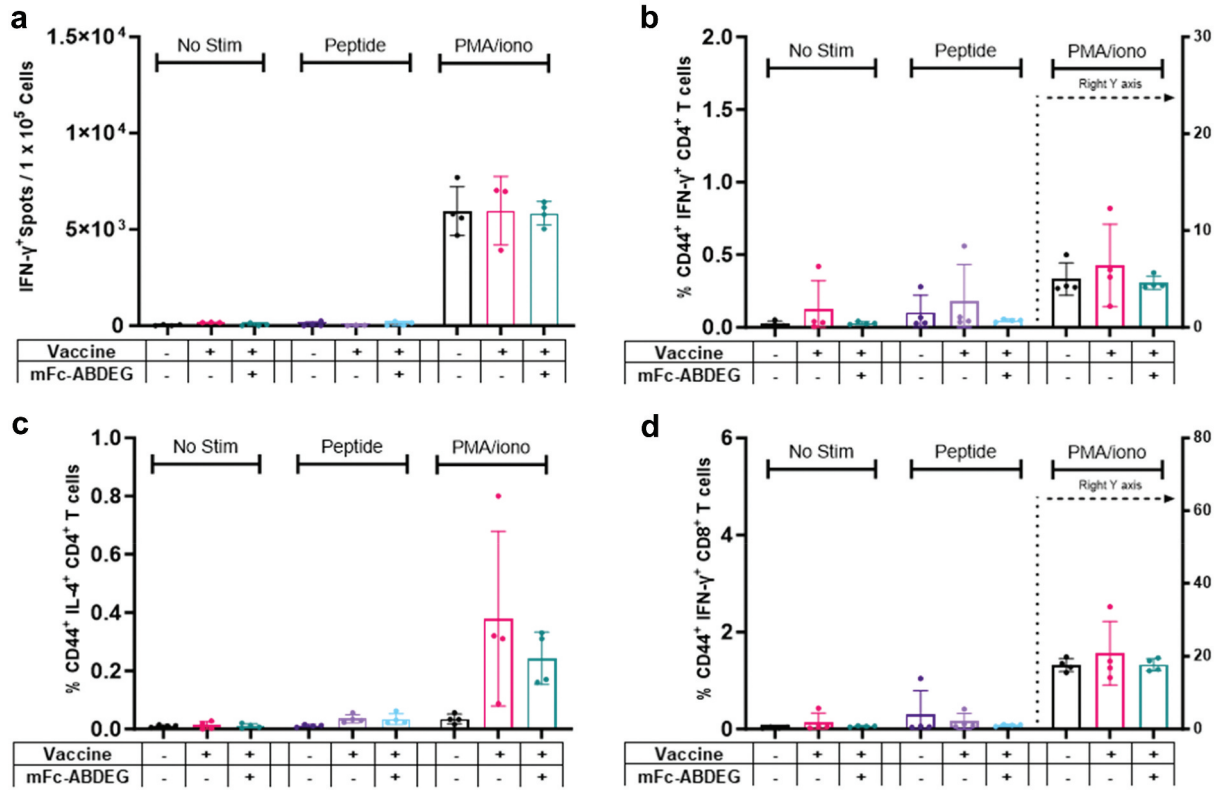


Figure 7. T cell responses to vaccination in mFc-ABDEG-treated animals (influenza model) (4 mice per group – 2 per sex): a) IFN-γ-secreting cells in vaccinated mice as measured by ELISPOT. Splenocytes were stimulated with an overlapping H1N1 peptide pool (miltenyi), PMA/ionomycin cocktail, or left unstimulated. b-d: H1N1-specific IFN-γ- and IL-4-secreting T cells at 10 days post-vaccination quantified by flow cytometry. T cells (live, singlet, CD3+, CD4+, or CD8+) after 6 h stimulation with a H1N1 peptide pool (miltenyi), PMA/ionomycin, or media (no stimulation) in the presence of protein transport inhibitor. Frequency of antigen-experienced (b) IFN-γ+ CD4 T cells, (c) IL-4+ CD4 T cells, and (d) IFN-γ+ CD8 T cells. Error bars represent standard deviation.

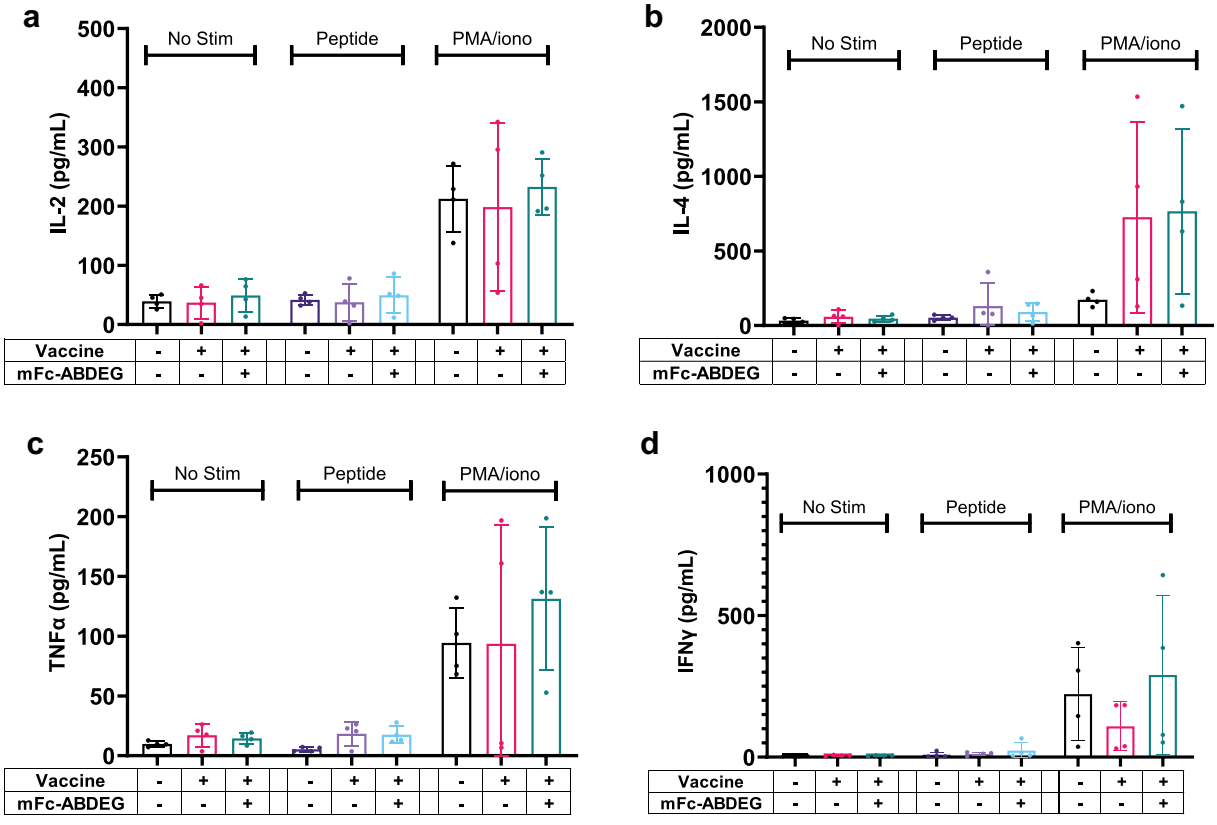


Figure 8. Cytokine levels in culture supernatant of restimulated splenocytes from mFc-abdeg-treated vaccinated animals (influenza) (4 mice per group – 2 per sex): splenocytes were stimulated with an overlapping H1N1-peptide pool (miltenyi), or PMA/ionomycin cocktail, or left unstimulated. Levels of T-cell associated cytokines IL-2 (a), IL-4 (b), IFN- γ (c) and TNFα (d) were measured in culture supernatant by multiplex cytokine ELISA. Error bars represent standard deviation.

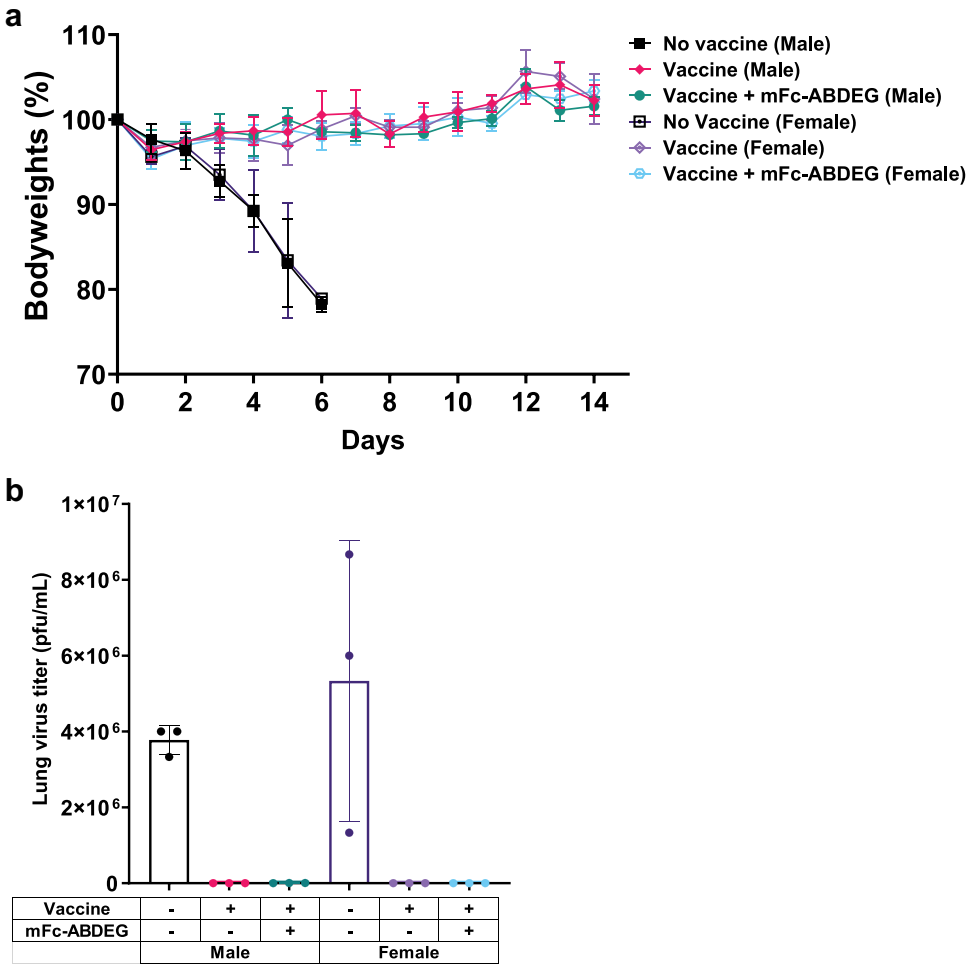


Figure 9. Vaccine-induced immunity protects mice against influenza infection irrespective of mFc-ABDEG treatment: (a) bodyweight changes in C57BL/6 mice challenged with IVR-180 h1N1 virus (18,000PFU) over a period of 14 days (12 mice per group – 6 per sex). (b) lung virus titers in male and female mice at 3 days post-infection (6 mice per group – 3 per sex). Error bars represent standard deviation.

compared to the vaccine-only group. These observations were seen for both COVID-19 and influenza models. Notably, a nonspecific increase in total IgG was observed in the unvaccinated PBS control group, which was likely triggered by a stimulation of the immune system secondary to a new housing environment.[32]

Discussion

Patients with autoimmune diseases exhibit an elevated susceptibility to infections because of their autoimmunity disorder, increased comorbidity, and treatment with immunosuppressive and immunomodulating drugs. The increased risk of infection e.g. with SARS-CoV-2, requires an effective vaccination to prevent severe disease outcomes. However, the efficacy of vaccines can be influenced by the concurrent use of immunosuppressive and immunomodulating drugs.[7–17] Indeed, drugs such as Prednisone, Janus kinase inhibitors, Methotrexate and Mycophenolate mofetil may attenuate the immune response elicited by vaccines, though they do not appear to induce non-response. Despite this, vaccination remains beneficial for patients. Similarly, TNF inhibitors, while not impairing a measurable immune response to vaccines, may still result in a less robust vaccine efficacy compared to healthy controls. Adjustments in dosing intervals or temporary suspension of medication may be considered for autoimmune patients undergoing immunosuppressive therapy.[7–14] B cell depleting therapy using anti-CD20 antibodies adversely impacts the production of protective antibodies by effector B cells and the generation of virus-specific memory B cells, crucial for antibody production upon re-exposure to antigens. The majority of individuals exhibit a robust CD4 and CD8 T cell response post SARS-CoV-2 infection or after vaccination, with some developing a memory phenotype potentially responsible for prolonged immunity. Unlike humoral responses, antigen-specific CD4 and CD8 T cell responses to vaccination remain unaffected by anti-CD20 therapy.[15–17] FcRn blockers decrease IgG levels to nadir levels that remain measurable and without adversely impacting the immune system mechanisms.[2] The transient nature of IgG reduction following FcRn inhibition indicates that B cell functionality, specifically IgG production by plasma cells, remains unaltered.[18] The safety of immunization with live or live-attenuated vaccines and the response to immunization with these vaccines during treatment with efgartigimod are unknown. For patients that are being treated with efgartigimod, vaccination with live or live-attenuated vaccines is generally not

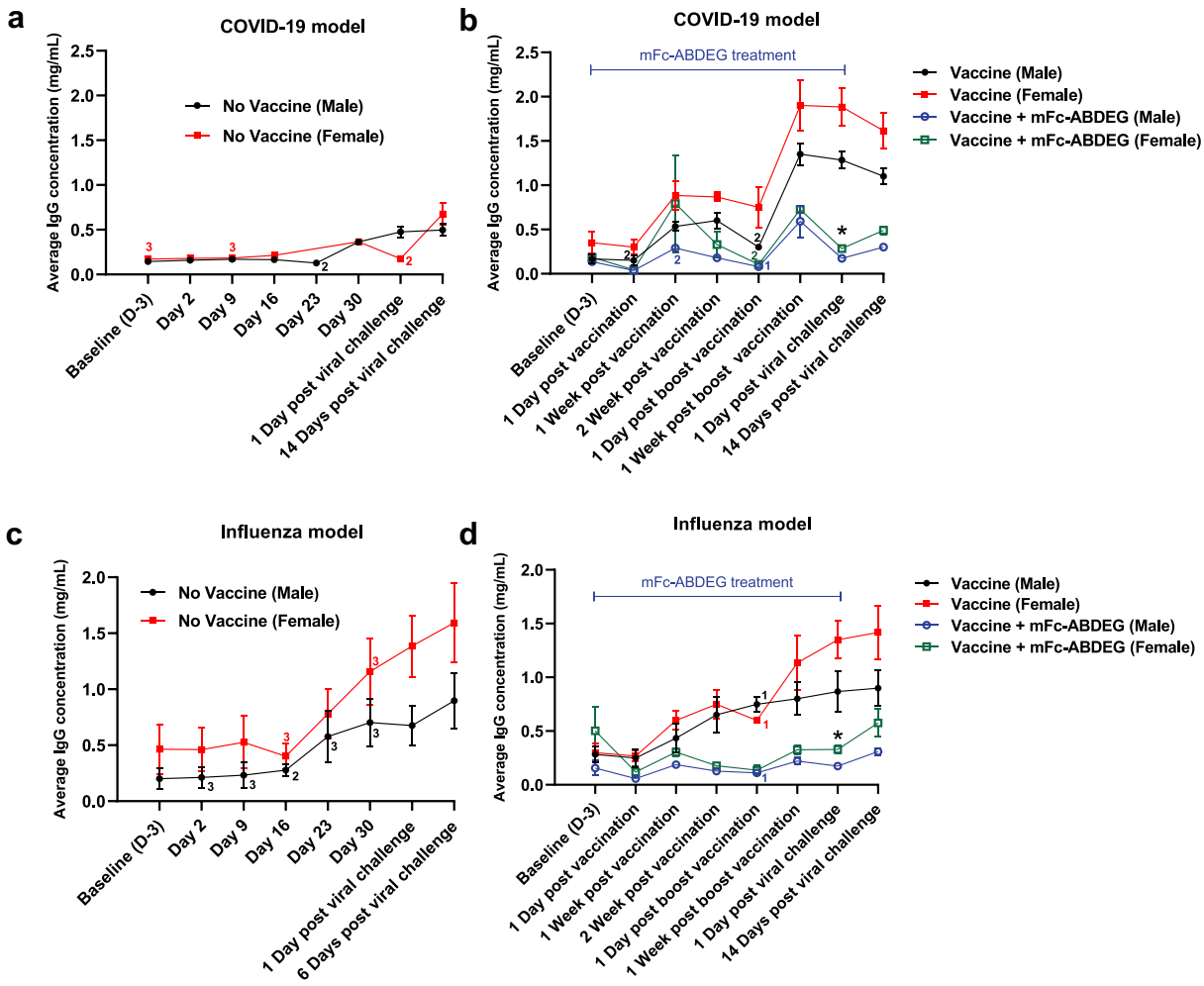


Figure 10. Total IgG concentration in unvaccinated mice and vaccinated mice with or without mFc-ABDEG treatment: ELISA was performed on all serum samples from each study group to assess the concentration of total IgG. (a) COVID-19 model: total IgG levels in unvaccinated PBS control group (4 mice per sex). (b) COVID-19 model: total IgG levels in vaccine-only (3 mice per sex) and vaccine + mFc-abdeg groups (3 mice per sex) (c) influenza model: total IgG levels in unvaccinated PBS control group (4 mice per sex) till day 6 post viral challenge, when all mice were euthanized as their body weight loss reached more than 20% of their initial body weight. (d) influenza model: total IgG levels in vaccine-only (3 mice per sex) and vaccine + mFc-ABDEG groups (3 mice per sex). Note: for a few timepoints, the numbers of actually analyzed mice are displayed. Error bars represent standard deviation.

recommended. If vaccination with live or live-attenuated vaccines is required, these vaccines should be administered at least 4 weeks before treatment and at least 2 weeks after the last dose of efgartigimod. Other vaccines may be administered as needed at any time during treatment with efgartigimod.[19] Clinical data confirmed that patients treated with efgartigimod can sustain the ability to mount humoral responses to vaccination, and levels of virus-specific antibody titers are maintained and closely follow the kinetics of total IgG reduction.[20–23]

In this study, we conducted more in-depth research into the impact of FcRn antagonism by an antibody Fc fragment (mFc-ABDEG), on vaccine-induced humoral and cellular responses along with its influence on protective immunity against viral challenges. The mFc-ABDEG is a mouse-adapted antibody Fc fragment closely emulating the functionality of efgartigimod. We used mouse vaccination models for both COVID-19 and influenza for this investigation. The mouse model used to study the effect of mFc-ABDEG treatment on effectiveness of vaccination to protect against a COVID-19 challenge did not yield high morbidity or mortality and therefore may not mirror human disease as observed early in the pandemic. However, observed lung pathology was reflective of COVID-19 related lung disease and therefore seems to be defendable as a model for the milder COVID-19 disease that was observed later in the pandemic.

In the herein presented COVID-19 study, lung pathology in female mice was similar to the one observed in male mice, however, virus titers recovered from lung tissues were lower at the time measured. It needs to be considered that peak virus titers may not coincide with the peak in pathology. Histopathological scoring can be high in the absence of detectable viral titers since scores are driven by the presence of immune cells, next to tissue damage markers like edema and necrotic cells. Although histopathological scores typically reflect virus-induced tissue damage, infiltrating immune cells can be part of the protective mechanism (for example cytotoxic T cells that help clear infected epithelial cells) or part of the active repair response after virus infection (M2 macrophages, eosinophils and Tregs). Hence, it is possible that histopathological scores reflect these mechanisms after the virus titers have peaked earlier or, in the case of a protective immune response, after virus titers were better controlled through

these mechanisms. We and others have observed sex differences in host responses to infection and vaccination, which may explain why peak virus titers might be at different timepoints post infection for male and female mice.

Vaccination induced robust humoral responses, as virus-specific binding and neutralizing antibodies were produced, even when animals were treated with mFc-ABDEG before and during the vaccination period. However, the titers of virus-specific IgG and total IgG were lower in mFc-ABDEG-treated mice, as expected, since mFc-ABDEG increases IgG degradation. In contrast, virus-specific IgM titers were not affected by mFc-ABDEG treatment, as IgM is not recycled by FcRn. The COMIRNATY vaccine induced a strong T cell (Th1) response, with reactive IFN-γ+ T cells against SARS-CoV-2 generated comparably in both mFc-ABDEG-treated and untreated vaccinated mice. Multiple assays used to evaluate T cell responses yielded consistent results, indicating that mFc-ABDEG treatment did not adversely affect T cell responses. QIV vaccination resulted in poor overall T cell induction, even with the use of AddaVax adjuvant, irrespective of mFc-ABDEG treatment. Poor T cell induction was anticipated due to the Th2-biased response that inactivated influenza vaccines typically induce.[31]

Interestingly, the mFc-ABDEG-mediated reduction of virus-specific IgG did not compromise protective immunity against viral challenges in both models. Mice treated with mFc-ABDEG were protected from pathology, body weight loss, and they controlled lung virus titers equally well as non-treated vaccinated controls. It is important to emphasize that in the influenza model, despite lower levels of virus-specific IgG and the lack of a measurable T cell response against the influenza virus, mice treated with mFc-ABDEG were all protected against viral challenge similarly to the non-treated vaccinated controls. This indicates that IgM and nadir-IgG levels were sufficient to control disease in these mice.

These findings align with the observations from a T-Cell – Dependent Antibody Response (TDAR) study conducted in Cynomolgus monkeys to examine the effect of efgartigimod treatment on humoral and cellular immune responses after immunization with Keyhole Limpet Hemocyanin (KLH).[33] Lower anti-KLH IgG titers were observed after the second KLH challenge in efgartigimod-treated animals, which normalized during the treatment-free period. No difference was observed in IgM titers or KLH-elicited cellular response (IFNγ ELISpot) between efgartigimod- and vehicle control-treated animals. The data showed that T-cell – dependent antibody and cellular immune responses were mounted to a prototypical antigen under efgartigimod treatment. Furthermore, in line with the mode of action, total IgG titers were significantly decreased under efgartigimod treatment and normalized during the treatment-free period. In addition, no difference was observed in antibody and cellular responses after a third KLH challenge during the treatment-free period between treated- and vehicle control animals, indicating that memory responses were not affected by efgartigimod treatment.

These non-clinical data further substantiate clinical observations from a controlled healthy volunteer vaccination study with efgartigimod: although IgG titers were reduced, FcRn antagonism with efgartigimod did not impair the ability to generate new specific IgG responses and B cell responses, regardless of the timing of a T cell independent vaccination in healthy volunteers (NCT05163834).[23] These results were further confirmed in a clinical setting in myasthenia gravis (MG) patients that were treated with efgartigimod IV or SC: MG patients who participated in MG studies ADAPT, ADAPT + or ADAPT-SC+ and that received influenza or COVID-19 vaccines (T-cell dependent vaccines) during treatment were able to mount antigen-specific humoral responses regardless of treatment with efgartigimod and even when total IgG levels were maximally suppressed.[20–22] In the controlled healthy volunteer vaccination study, the established safety profile of efgartigimod was maintained, and there was no increased risk of infections. Similar observations were reported in MG patients who received vaccinations during the ADAPT, ADAPT+, and ADAPT-SC+ studies. Based on the clinical safety and immune response assessments, vaccines, except live or live-attenuated vaccines, may be administered as needed at any time during treatment with efgartigimod.[19]

Although vaccine efficacy against viral challenge was not affected by the reduction in antibody levels, it is important to note that the animals were challenged 10 days after the boost vaccination. At this time, antibody levels are expected to be relatively high compared to real-world situations, where antibody levels gradually decline over time, and memory responses play a more significant role in viral protection.[34–36] Nevertheless, this study assessed the most extreme condition, characterized by maximum antibody reduction by mFc-ABDEG during both vaccination and viral challenge. In real-world situations, persistent memory responses are crucial for protection against infections. As previously discussed, efgartigimod does not impair the reactivation of memory cells, as demonstrated in the TDAR study. Additionally, in the phase 2 trial with efgartigimod in pemphigus patients, preexisting autoantibodies (anti-varicella zoster virus (VZV), anti-pneumococcal capsular polysaccharide (PCP) and anti-tetanus toxin (TT) antibodies) were measured. Upon efgartigimod treatment, anti-VZV and anti-PCP antibodies reached nadir between Days 22 and 36, whereas anti-TT IgG exhibited a somewhat slower decline. Total IgG and evaluated antibody titers (anti-VZV, anti-PCP, anti-TT) remained stably suppressed for as long as patients were dosed with biweekly efgartigimod. Following the last infusion (Day 238), a gradual return of total IgG and evaluated antibodies to baseline levels was observed. The subsequent return to normal levels upon cessation of efgartigimod treatment indicated no negative effect on long-lived plasma cell (LLPC) responses.[20] The viral challenge data further supports that protective antibody levels from vaccination can be preserved, even when total IgG levels are reduced to nadir levels by efgartigimod. These results are translatable to the human situation in principle, though, in humans immune responses elicited by vaccination can vary based on preexisting immunity, concomitant immunosuppressive therapies and the presence of chronic underlying conditions, as well as the age and sex of the patients, while in the presented controlled animal studies de novo immune responses were assessed without studying the impact of the cited factors.

In conclusion, mFc-ABDEG treatment did not affect the generation of protective vaccine-induced immune responses in both COVID-19 and influenza vaccination models. mFc-

ABDEG treatment reduced the levels of virus-binding and neutralizing IgG, but not IgM, in line with the mode of action of mFc-ABDEG. Protective immunity against viral challenge was fully maintained in both models despite the lowered anti-virus IgG titers caused by mFc-ABDEG treatment. Findings from these vaccination studies and previous observations indicate that FcRn antagonism does not interfere with the underlying mechanisms of the immune system's response to vaccination. By comparison, immunosuppressive or immunomodulating agents adversely affect the essential immune components needed to generate vaccine-induced responses. FcRn antagonism selectively targets IgG recycling in a transient manner, without impacting IgG production by plasma cells or cellular activity.

Disclosure statement

The M.S. laboratory has received unrelated research funding in sponsored research agreements from Moderna, 7Hills Pharma and Phio Pharmaceuticals which has no competing interest with this work.

All authors declare no other competing interests except that MM, OB, HW, PV, MN, NE, KA, SS, PU, JB are or were employees of argenx and hold argenx stock options.

GS, PW, LC, GL, MS are or were employees of Icahn School of Medicine at Mount Sinai at the time of study.

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Sophie Steeland is a leading principal clinical scientist for efgartigimod at argenx. Her research focuses on clinical development of therapeutic antibodies for treatment of autoimmune diseases. With a strong background in clinical research and a particular interest in immunology and neurology, she is dedicated to ensuring that promising antibody therapies are effectively developed for patient use. She was scientific lead on the MG program of efgartigimod and supported regulatory approval of Vyvgart and Vyvgart hytrulo in MG in multiple regions.

Peter Ulrichts is the Chief Scientific Officer at argenx. His innovative work has contributed to the development of various therapeutic antibodies for the treatment of cancer and autoimmune diseases. He headed the development of argenx' FcRn antagonist efgartigimod until the first-in-human study, after which he transitioned to become the lead scientist of the efgartigimod program. In his current role, he oversees the development of all clinical and pre-clinical compounds within argenx's pipeline.

Judith Baumeister is the Head of Toxicology & Non-Clinical Pharmacology at argenx, with 30 years of experience in drug safety and efficacy evaluation. Prior to argenx she has worked in academia and industry on virology, vaccines and biotherapeutics. Key activity and expertise are nonclinical safety of biotherapeutics, physiology of

nonclinical species, responses to vaccines. She has been a key contributor to regulatory submissions for efgartigimod.

Michael Schotsaert is an immunologist and vaccinologist specializing in influenza, ZIKA, and SARS-CoV-2. With over 20 years of experience in host-pathogen interactions, he develops and evaluates vaccines, adjuvants, and antiviral treatments using preclinical infection models. Since 2020, he has led a research team at the Icahn School of Medicine at Mount Sinai, studying immune responses to infection and vaccination, particularly in the context of comorbidities like obesity, diabetes, and aging.

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Authors' contributions

PW, GS, MS, MM, OB, and JB designed the study. PW, GS, and LAC conducted animal models and sample analysis. PW, GS, LAC, MS, MM, and OB performed data analyses and interpretation. PV, MN, NE, and KA contributed to IgG sample analysis and data interpretation. HW conducted statistical analysis and interpretation. PW, GS, LAC, MS, and MM wrote the initial draft of the manuscript. PW, GS, LAC, MS, MM, OB, SS, HW, PV, PU, and JB contributed to the writing, and all authors reviewed the manuscript.

Biographical note

The Schotsaert laboratory uses viruses, adjuvants and vaccines to study host immune responses to infection and vaccination in preclinical models. The outcome of the Schotsaert lab research contributes to a better understanding of host-pathogen interactions and is important for adjuvant and vaccine development, and to better understand the impact of immunological or antiviral interventions on host immunity.

argenx* is a global immunology company committed to improving the lives of people suffering from severe autoimmune diseases. Partnering with leading academic researchers through its Immunology Innovation Program (IIP), argenx aims to translate immunology breakthroughs into a world-class portfolio of novel antibody-based medicines. argenx developed and is commercializing the first approved neonatal Fc receptor (FcRn) blocker, globally in the U.S., Japan, the EU, the UK, China and Canada. The Company is evaluating efgartigimod in multiple serious autoimmune diseases and advancing several earlier stage experimental medicines within its therapeutic franchises.

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Ethics

The animal studies were reviewed and approved by IACUC of the Icahn School of Medicine at Mount Sinai.

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