Post-vaccination IgG4 and IgG2 class switch associates with increased risk of SARS-CoV-2 infections

Carla Martín Pérez, Sílvia Ruiz-Rius, Anna Ramírez-Morros, Marta Vidal, D. Herbert Opi, Pere Santamaria, Julià Blanco, Josep Vidal-Alaball, James G. Beeson, Luis M. Molinos-Albert, Ruth Aguilar, Anna Ruiz-Comellas, Gemma Moncunill, Carlota Dobaño

PII: S0163-4453(25)00067-2

DOI: https://doi.org/10.1016/j.jinf.2025.106473

Reference: YJINF106473

To appear in: *Journal of Infection*
Accepted date: 12 March 2025

Please cite this article as: Carla Martín Pérez, Sílvia Ruiz-Rius, Anna Ramírez-Morros, Marta Vidal, D. Herbert Opi, Pere Santamaria, Julià Blanco, Josep Vidal-Alaball, James G. Beeson, Luis M. Molinos-Albert, Ruth Aguilar, Anna Ruiz-Comellas, Gemma Moncunill and Carlota Dobaño, Post-vaccination IgG4 and IgG2 class switch associates with increased risk of SARS-CoV-2 infections, *Journal of Infection,* (2025) doi:https://doi.org/10.1016/j.jinf.2025.106473

Post-vaccination IgG4 and IgG2 class switch associates with increased risk of SARS-CoV-2 infections

Carla Martín Pérez [1,2*], Sílvia Ruiz-Rius [1,2*], Anna Ramírez-Morros [3], Marta Vidal [1,2], D. Herbert Opi [4,5,6], Pere Santamaria [7,8], Julià Blanco [9,10,11,12], Josep Vidal-Alaball [3,11,13], James G. Beeson [4,5,6], Luis M. Molinos-Albert [1,2], Ruth Aguilar [1,2], Anna Ruiz-Comellas [3,11,13,14*], Gemma Moncunill [1,2,10*], Carlota Dobaño [1,2,10*]

Affiliations

[1] ISGlobal, Barcelona, Catalonia, Spain.

[2] Facultat de Medicina i Ciències de la Salut, Universitat de Barcelona (UB), Barcelona, Spain.

[3] Unitat de Suport a la Recerca de la Catalunya Central, Fundació Institut Universitari per a la Recerca a l'Atenció Primària de Salut Jordi Gol i Gurina, Manresa, Spain.

[4] Burnet Institute, Melbourne, Australia.

[5] Department of Infectious Diseases, University of Melbourne, Australia

[6] School of Translational Medicine, Monash University, Australia

[7] Institut d'Investigacions Biomèdiques August Pi Sunyer, Barcelona, Spain.

[8] Department of Microbiology, Immunology and Infectious Diseases, Snyder Institute for Chronic Diseases, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada.

[9] IrsiCaixa, Badalona, Spain.

[10] CIBER de Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, Barcelona, Spain.

[11] Department of Medicine. Faculty of Medicine. Universitat de Vic-Central de Catalunya, UVic-UCC, Vic, Spain.

[12] Institut Germans Trias I Pujol, IGTP, Badalona, Spain.

[13] Health Promotion in Rural Areas Research Group, Gerència d'Atenció Primària i a la Comunitat Catalunya Central, Institut Català de la Salut, Manresa, Spain.

[14] Centre d'Atenció Primària (CAP) Sant Joan de Vilatorrada, Gerència d'Atenció Primària i a la Comunitat Catalunya Central, Institut Català de la Salut, Spain.

* Shared authorships

Corresponding authors:

Carlota Dobaño, Research Professor
carlota.dobano@isglobal.org
ISGlobal
Carrer del Rosselló, 153, 08036 Barcelona, Spain.
+34 93 2275400 ext. 4519

Gemma Moncunill, Assistant research Professor
gemma.moncunill@isglobal.org
ISGlobal
Carrer del Rosselló, 153, 08036 Barcelona, Spain.
+34 93 2275400 ext. 4519

Abstract

Objectives

Repeated COVID-19 mRNA vaccinations increase SARS-CoV-2 IgG4 antibodies, indicating extensive IgG class switching following the first booster dose. This shift in IgG subclasses raises concerns due to the limited ability of IgG4 to mediate Fc-dependent effector functions.

Methods

To assess the impact of IgG4 induction on protective immunity, we analyzed longitudinal SARS-CoV-2 IgG subclasses, C1q and FcγR responses, and neutralizing activity in a well-characterized cohort of healthcare workers in Spain.

Results

Elevated IgG4 levels and higher ratios of non-cytophilic to cytophilic antibodies after booster vaccination were significantly associated with an increased risk of breakthrough infections (IgG4 HR[10-fold increase]=1.8, 95% CI=1.2–2.7; non-cytophilic to cytophilic ratio HR[10-fold increase]=1.5, 95% CI=1.1–1.9). Moreover, an increased non-cytophilic to cytophilic antibody ratio correlated with reduced functionality, including neutralization.

Conclusions

These findings suggest a potential association between IgG4 induction by mRNA vaccination and a higher risk of breakthrough infection, warranting further investigation into vaccination strategies to ensure sustained protection.

Keywords

SARS-CoV-2, humoral immunity, COVID-19 mRNA vaccines, SARS-CoV-2-specific antibodies, Fc effector function

Introduction

Throughout the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, a variety of vaccines targeting the viral spike (S) protein have been developed, with the mRNA formulated BNT162b2 and mRNA-1273 leading the way in Western countries, alongside replication-deficient adenovirus-based vaccines. Despite the ability of mRNA vaccines to induce strong and durable immune responses, emerging evidence has raised questions regarding antibody class switching[1,2]. Several reports suggest that repeated doses of COVID-19 mRNA vaccines lead to a shift toward IgG4 antibodies, and an expansion of memory B cells expressing IgG4[1,3,4], several months after the primary series, with further increases following a first or second booster[1,2,5–10]. Such IgG4 induction has not been reported following vaccination with adenoviral vector-based[1] or other COVID-19 vaccines[7,11].

This IgG subclass evolution has raised concerns due to the limited capacity of IgG4 to mediate Fc-dependent effector functions in cooperation with innate immune cells, such as antibody-dependent cellular phagocytosis, cytotoxicity, or to fix complement[12,13]. IgG4 is primarily involved in regulatory functions, and is associated with immune tolerance and chronic antigen exposure[14]. These contrast with the pro-inflammatory related roles of IgG1 and IgG3, which are more efficient at engaging

complement and/or Fc gamma receptors (FcγR) on the surface of monocytes, neutrophils or natural killer cells[12]. IgG2 is usually involved in responses against polysaccharide antigens, and has limited binding to FcγRs, resulting in lower effector functions[12]. In addition, IgG1 and IgG3 also exhibit superior neutralizing capacity, further distinguishing them from IgG2, and IgG4[12]. Although the reduced Fc-dependent function of IgG4 may compromise the antibody-mediated effector immune response, the impact of IgG4 class switch on protection against SARS-CoV-2 infection remains unclear, and the contribution of IgG2 has received less attention. This study investigated the consequences of IgG4 induction associated with repeated mRNA COVID-19 vaccinations on protective immunity against SARS-CoV-2 infection, which is essential for monitoring the long-term efficacy of vaccines, particularly as booster doses are regularly administered.

Materials and Methods

Study design and setting

This study included a longitudinal subset of primary healthcare workers (HCW) from the ongoing CovidCatCentral cohort recruited from three counties in Barcelona province, Spain (**Fig. 1, 2**). A first group of HCW was enrolled during the first wave of the COVID-19 pandemic (cohort A Fig. 2, March–April 2020, n=247) among all those who had a symptomatic SARS-CoV-2 infection confirmed by rRT-PCR and/or rapid diagnostic tests (RDT). All HCWs with COVID-19 were invited to participate. Questionnaires and venous blood samples were collected at thirteen cross-sectional surveys up to the end of May 2024. A second group included naïve HCWs recruited March–April 2021 after completing full primary vaccination (cohort B Fig. 2, n=200). This group was selected to have similar characteristics (age, sex, professional category, smoking habits) to the pre-exposed group. These HCWs were visited at nine cross-sectional surveys, with venous blood samples collected up to the end of May 2024. Demographic and clinical data were collected at baseline and during follow-up visits through telephone interviews and electronic questionnaires conducted by study physicians and nurses. The recorded information included confirmed SARS-CoV-2 infections, COVID-19 symptoms, vaccination type, dates and adverse effects, comorbidities and smoking status. In this study, a longitudinal set of HCW from cohorts A and B, were randomly selected among those with 3 doses of vaccination at T9, and available samples were included for the kinetics analysis (n=83) (**Fig. 2**). Among the 83 participants in the longitudinal analysis, 27 received a fourth dose between T9 and T11, and 7 received a fifth dose between T11 and T13. An additional set of 66 HCW from cohorts A and B, who had received 3 vaccine doses (up to timepoint 9 visit) were analyzed only at the timepoint (T) 9 visit corresponding to May 2022, 4 months after the first booster vaccination to increase statistical power to evaluate correlates of protection against SARS-CoV-2 infection in the following 6 months, when BA.5 and BQ.1 Omicron variants were dominant. All breakthrough infections observed during the study follow-up period were mild, precluding the assessment of the impact of disease severity on the analyzed outcomes. Baseline characteristics of participants included in the correlates of protection analysis are shown in **Table 1**. Plasma analyzed in this study corresponded to blood samples collected at the following timepoint visits: pre-vaccination at T0 (a baseline sample obtained July–September 2020 from the VisCat study survey involving all HCW in Catalonia), T1 (September 2020), T2 (October 2020), T3 (November 2020), T4 (January-February 2021); and post-vaccination T5 (March–April 2021), T7 (July 2021), T9 (May 2022), T10 (November-December 2022), T11 (May-June 2023), and T13 (May 2024). Diagnosed infections (typically symptomatic) were identified through passive case detection, meaning participants underwent testing by rRT-PCR and/or rapid diagnostic tests (RDT) based on the presence

of symptoms or known exposure to an infected individual. Undiagnosed infections (typically asymptomatic) were identified through serology, as previously described[15].

The study protocols were approved by the IRB Comitè Ètic d'Investigació Clínica IDIAP Jordi Gol (code 20/162-PCV), and written informed consent was obtained from all study participants before enrollment.

Our study included both males and female, although the latter were predominant, and no sex-based differences were observed in the findings.

Quantification of IgG subclasses

Levels of IgG1-4 to the full-length SARS-CoV-2 nucleocapsid (N) and S antigen, and the receptor binding domain (RBD) from Wuhan and viral variants (Delta, BA.1.1, BA.2, BA.4/5, and BQ.1.1), were measured as median fluorescence intensity (MFI) by quantitative suspension array technology[16]. Plasma samples (1:500 for IgG1 and 1:100 dilution for IgG2, IgG3 and IgG4) were incubated in 384-well plates with multiplexed MagPlex-C beads previously coupled to the SARS-CoV-2 proteins at a concentration of 10,000 beads/µl (1500 beads/well) for 1 h at room temperature (RT), protected from light and with 900 rpm agitation. A positive control (pooled plasma from 30 exposed and vaccinated volunteers) serially diluted, pre pandemic negative controls from healthy individuals (n=129), and technical blanks (Luminex buffer), were added alongside test samples. Semi-automatic Viaflo and Assist Plus Integra devices were used for liquid handling. After washing the plates three times with PBS-T (0.05% Tween 20 in phosphate-buffered saline [PBS]) using a BioTek 405 TS washer, samples were incubated for 30 min at RT and 900 rpm with the respective mouse anti-human IgG subclass secondary antibodies conjugated to phycoerythrin (PE) at the appropriate dilutions (IgG1 at 1:250 and IgG2-4 at 1:50, 2µg/ml, Southern Biotech). After washing the plates three times, the bead-bound complexes were resuspended in Luminex Buffer and antibody measures (MFI) were read using a Flexmap 3D® (xMAP, Luminex).

RBD and Spike Wuhan proteins were expressed in lentiviral-transduced CHO-S cells and Strep tag-purified (obtained from the Santamaria Lab, IDIBAPS). RBD from variants of concern (Delta, BA1.1, BA.2, BA.4/5, BQ.1.1 and XBB) were produced in eukaryotic cells in house.

Quantification of FcγR-binding antibodies

Binding of N, S and RBD SARS-CoV-2 specific antibodies to FcγR (I, IIa, IIb, IIIa, IIIb)[17] were measured as MFI by quantitative suspension array technology. Plasma samples (1:200 dilution) alongside positive and negative controls were incubated with multiplexed beads coupled to SARS-CoV-2 proteins in 384-well plates (1500 beads/well) for 2 h at RT and 900 rpm. After washing plates three times with PBS-Tween, bead-bound complexes were incubated for 1 h at RT with biotin-conjugated FcγRs in Luminex Buffer previously mixed with streptavidin-PE (1:250 dilution). The different receptors used were either FcγRI (0.25 µg/µl), FcγRIIa H131 (0.88 µg/µl), FcγRIIb (1.12 µg/µl), FcγRIIIa V158 (0.92 µg/µl), or FcγRIIIb (0.92 µg/µl) (produced at Duke Institute facility, USA), all at a final concentration of 0.5 µg/ml. After washing the plates, the microspheres were resuspended in 100 µl Luminex Buffer and antibody measures read using Flexmap 3D®. A positive control (pooled plasma from 30 exposed and vaccinated volunteers) serially diluted, and technical blanks (Luminex buffer), were added alongside test samples.

Quantification of C1q-binding antibodies

Binding of N, S and RBD SARS-CoV-2 specific antibodies to C1q[13] were measured as MFI by quantitative suspension array technology. Plasma samples (1:200 dilution) alongside positive and negative controls were incubated with multiplexed beads coupled to SARS-CoV-2 proteins in 384-well plates (1500 beads/well) for 30 min at RT and 900 rpm. After washing plates three times with PBS-Tween, bead-bound complexes were incubated for 30 min with C1q (1 µg/µl, Complement C1q Human, EMD Millipore Corp.) at 0.0015625 µg/µl diluted in Luminex Buffer. After washing, samples were incubated with rabbit anti-human C1q IgG (1:250 dilution, 9.1mg/ml, provided by Beeson J.G. lab) for 15 min, washed again, and incubated for 15 min with PE-conjugated secondary antibody goat anti-rabbit IgG (1:250 dilution, Thermo Fisher Scientific). After washing the plates, the microspheres were resuspended in Luminex Buffer and antibody measures read using Flexmap 3D®. A positive control (pooled plasma from 30 exposed and vaccinated volunteers) serially diluted, pre pandemic negative controls from healthy individuals (n=129), and technical blanks (Luminex buffer), were added alongside test samples.

Neutralizing activity of plasma

A standardized pseudovirus-based neutralization assay was conducted using HIV reporter pseudoviruses that express the SARS-CoV-2 S protein, along with Luciferase, as described previously[18]. The assay was carried out in duplicate. Briefly, 200 TCID50 pseudoviruses were preincubated with heat-inactivated plasma samples, serially diluted three-fold (1/60–1/14), at 37 °C for 1 h in Nunc 96-well cell culture plates (Thermo Fisher Scientific, USA). Subsequently, 2x10^4 HEK293T/hACE2 cells treated with DEAE-Dextran (Sigma-Aldrich, USA) were added. After 48 h, results were measured using the EnSight Multimode Plate Reader and BriteLite Plus Luciferase reagent (PerkinElmer, USA). The values were normalized, and the ID50 (the reciprocal dilution inhibiting 50% of the infection) was calculated by plotting the log of plasma dilution versus response, and fitting it to a four-parameter equation in Prism 8.4.3 (GraphPad Software, USA). Neutralizing activity was reported as *IC50* neutralization titers.

Statistical analysis

IgG subclass and C1q-binding antibody levels were $\log_{10}$-transformed. The difference in antibody levels between consecutive timepoints was measured by Wilcoxon Signed-Rank test, and the associations between immune parameters by Spearman's rank correlation, both adjusted by Benjamini-Hochberg to account for multiple testing. To investigate the effect of antibody levels on the risk of SARS-CoV-2 breakthrough infection among individuals with a confirmed infection date (referred to as symptomatic, n = 52), we conducted a survival analysis using Kaplan–Meier and multivariable Cox regression modeling. Vaccinated individuals were considered at risk from T9 until the first reported episode of SARS-CoV-2 breakthrough infection, receipt of an additional vaccine dose (at which point they were censored), or the last day of study follow-up (T10). Participants with asymptomatic infections (determined by serology) during the follow-up period were excluded from the Kaplan–Meier and Cox regression analysis, as the timing of those infections could not be ascertained. To elucidate the effect of antibody levels on the risk of SARS-CoV-2 breakthrough infection in individuals with either symptomatic (with a known infection date) or asymptomatic (not known infection date) breakthrough infection events (n = 58), we performed multivariable logistic regression modeling. In these analyses, vaccinated individuals were considered infected during the follow-up period if they experienced an infection without subsequent vaccination during the same period or were infected prior to receiving

an additional vaccine dose (for those with diagnosed infections). Conversely, participants who did not experience an infection during the follow-up period were considered uninfected. Participants were excluded from the logistic regression analysis if they were uninfected and vaccinated during the follow-up period, had an asymptomatic infection, and were vaccinated during the same follow-up period, or were diagnosed infected after receiving a vaccine dose within the same period. Models were adjusted by age, sex, number of infections, time since last exposure comorbidities, and smoking. P-values lower than 0.05 were considered statistically significant. All tests were performed two-sided. Statistical analysis was done in R version 4.2.2.

Results

Kinetics of IgG subclasses, and FcγR and C1q-binding antibodies

To understand how repeated mRNA vaccinations shape the dynamics of antibody responses over time, we longitudinally evaluated the kinetics of IgG subclasses binding SARS-CoV-2 antigens, over a period of nearly three years after the first booster dose (**Fig. 3a**, **Table 2, Suppl Fig. 1, 2**). Given that cytophilic antibodies (IgG1, IgG3) mediate stronger Fc-dependent effector functions than non-cytophilic antibodies (IgG2, IgG4), we also assessed the kinetics of FcγR and C1q-binding antibodies, which serve as a surrogate for Fc-mediated effector functions[19], over the same time frame (**Fig. 3a**, **Table 2**), and their correlations 4 months post-booster (**Fig. 3b,c, Suppl Fig. 3**). IgG1, IgG2, IgG4, as well as FcγR and C1q-binding antibody levels, showed a significant increase following both the primary vaccination and the first booster dose, after which they stabilized (**Fig. 3a**, **Table 2**). The ratio of IgG4 to total IgG also increased from 2 months post-primary vaccination to 5 months post-primary vaccination, and then stabilized (**Suppl Fig. 4**). In contrast, IgG3 levels rose sharply after the primary vaccination but began to decline by 4 months post-vaccination, eventually returning to baseline levels (**Fig. 3a**, **Table 2**).

Correlation of the ratio of non-cytophilic to cytophilic antibodies with antibody functionality

At the individual level, we detected a significant inverse correlation between the ratio of non-cytophilic to cytophilic antibodies and C1q and FcγR-binding antibody levels and neutralization 4 months post-booster (**Fig. 3b,c, Suppl Fig. 3, 5**). Moreover, IgG2 and IgG4 showed a negative correlation with FcRγIIIb-binding antibody levels and neutralization activity, while IgG1 was positively associated with C1q and FcγR-binding antibody levels as well as with neutralization capacity. These results suggest that the expansion of non-cytophilic relative to cytophilic IgG antibodies is associated with reduced antibody functionality.

Association of IgG subclasses and ratio of non-cytophilic to cytophilic antibodies with risk of SARS-COV-2 infection

To understand the implications of this antibody shift on protection, we first analyzed the effect of antibody levels post-booster vaccination on COVID-19 over a 6-month follow-up period (June 2022–December 2022). A higher ratio of IgG4 and IgG2 relative to IgG1 and IgG3 was significantly associated with an increased risk of symptomatic disease in univariable time-to-event analysis (**Fig. 4a, Suppl Fig. 6**) and multivariable models (**Fig. b, Suppl Table 1, Suppl Fig. 7**). In contrast, higher neutralizing activity (HR[10-fold increase]=0.36, 95% CI=0.16–0.85, p=0.019) and C1q and FcγIIIbR-binding antibody levels were significantly associated with a reduced risk of COVID-19 (**Fig. 4a,b,c**). In addition, we analyzed the association between antibody levels post-booster vaccination with protection against symptomatic and asymptomatic infections together over the same 6-month follow-up period. Higher levels of anti-

RBD antibodies binding to FcγRIIa, FcγRIIIa, and FcγRIIIb, and of anti-S antibodies binding to FcγRIIIa and FcγRIIIb (**Fig. 4c**), as well as C1q-binding antibodies to RBD and S (**Fig. 4b**), were linked to a reduced risk of infection. Moreover, elevated levels of IgG1 to S and RBD, and IgG3 to RBD from the Omicron BQ.1.1 variant, also predicted protection (**Fig. 4b, Suppl Fig. 7**). Conversely, higher levels of IgG2 to ancestral S and RBD, and IgG4 to ancestral S, RBD, and BQ.1.1 RBD, predicted risk of infection. Consistently, higher ratios of non-cytophilic to cytophilic antibodies to ancestral S, RBD, and BQ.1.1 RBD, were associated with a higher risk of SARS-CoV-2 infection (**Fig. 4b**). Importantly, when we adjusted the Cox and Logistic regression models assessing the impact of IgG4 levels on breakthrough infection by IgG1 levels, the association between IgG4 levels and risk of infection remained significant, indicating that IgG4 associates with increased risk independent of IgG1 levels (**Suppl Fig. 8**).

Discussion

Here, we show that higher levels of IgG4 and IgG2, as well as higher proportions of non-cytophilic to cytophilic antibodies, following booster vaccination, are associated with a heightened risk of SARS-CoV-2 breakthrough infection. Conversely, IgG1 levels, C1q- and Fcγ receptor-binding antibodies and neutralization capacity are associated with protection.

Our study aligns with previous research showing a sharp increase in IgG4 and IgG2 levels following three doses of mRNA vaccination against SARS-CoV-2[1,2], but goes beyond the state-of-the-art by associating this switch with decreased neutralization, Fc-effector functionality, and protective immunity. Nevertheless, while our findings indicate that higher IgG4 and IgG2 levels are associated with an increased risk of SARS-CoV-2 breakthrough infection, these less immune-activating subclasses may also help prevent severe COVID-19 by mitigating inflammation-driven pathology[20]. Previous studies have also reported negative correlations between Fc-effector functions and IgG4 induction. For instance, ADCP phagocytosis scores and ADCD were reduced after the third mRNA vaccine dose compared to the second dose, with these reductions correlating with increased anti-S IgG4 levels[1]. Similarly, a higher anti-S IgG4/IgG1 ratio after SARS-CoV-2 mRNA vaccination was associated with diminished NK cell activation and ADCD[6]. Beyond its reduced ability to engage effector functions, IgG4 is functionally monovalent due to its capacity for Fab-arm exchange[21], which may limit its ability to form immune complexes and effectively neutralize pathogens.

Both IgG2 and IgG4 B-cells typically accumulate high levels of somatic hypermutations, indicative of extensive affinity maturation. This suggests that antibodies of these subclasses may exhibit high binding affinities for their target antigens, which is generally important for neutralization. However, despite this potential for high affinity, we observed a negative association between IgG2 and IgG4 levels and neutralizing antibody responses.

The underlying mechanisms driving class-switch recombination towards IgG4, especially after repeated COVID-19 mRNA vaccination, are still unclear. It has been hypothesized that persistent germinal center responses induced by mRNA vaccination[22]—possibly driven by the prolonged presence of vaccine mRNA or antigen in lymph nodes[23]—may facilitate class switching toward distal subclasses such as IgG4[2]. This class switch toward IgG4 has also been observed following repeated vaccinations against malaria, HIV, and pertussis[24–26]. In malaria, elevated IgG4 levels and a higher ratio of non-cytophilic to cytophilic IgG subclasses were also associated with an increased risk of malaria after RTS,S vaccination[27]. Additionally, IgG4 targeting the circumsporozoite protein of the malaria parasite was found to interfere with opsonic phagocytosis, effectively inhibiting this critical immune process[28].

Similarly, in HIV-vaccinated individuals, the depletion of IgG4 antibodies from sera led to a significant enhancement in Fc-mediated effector functions[25].

Increasing evidence indicates that repeated mRNA SARS-CoV-2 vaccination is associated with sharp increases in anti-spike IgG4[1,2,5–7,9,10]. However, the induction of IgG4 by other vaccine platforms remains less clear. Adenoviral-based SARS-CoV-2 vaccines, as well as natural infection, have not been shown to trigger IgG4 class switching[3,10,29,30]. In contrast, the potential for inactivated and protein-based SARS-CoV-2 vaccines to induce IgG4 remains uncertain, with conflicting reports[7,31–33]. Moreover, while infections alone have not been associated with IgG4 induction, one study found that breakthrough infections occurring three months after primary mRNA vaccination —though not those within the first two months— did result in elevated IgG4 levels[1]. Interestingly, the sequence of infection relative to mRNA vaccination appears to influence the IgG4 response, with a study reporting that individuals who had a prior infection before vaccination exhibit lower IgG4 levels after vaccination compared to those without prior infection[10]. Since our study focused on IgG4 responses to mRNA vaccination, further studies should explore whether IgG4 induced by alternative vaccination strategies or breakthrough infections show similar associations with increased risk.

Our study has some limitations. First, our cohort primarily consisted of young adult women from the healthcare worker population, which may not fully reflect the broader population and restricts the power to compare sex-based differences. Second, we measured C1q- and FcγR-binding antibodies rather than directly assessing complement deposition or Fc effector cellular functions. However, FcγR-binding antibody levels have been established as reliable surrogates for mimicking FcγR engagement and have shown strong correlations with in vitro Fc effector assays, including antibody-dependent cellular cytotoxicity and phagocytosis[19]. Additionally, C1q binding has been shown to correlate with in vitro antibody-dependent complement deposition assays[34]. Although IgM contributes to complement activation, IgM levels in our study were low, suggesting that its influence on C1q binding is likely minimal. Furthermore, our cohort includes participants both with and without a SARS-CoV-2 infection prior to vaccination, which may introduce variability in immune responses and infection risk. Nevertheless, this has been accounted for in the correlates of protection analysis, as having an infection prior to vaccination was included as a covariate in both the Cox and logistic regression models. Although our study suggests IgG4 levels increase with repeated mRNA vaccine doses and higher IgG4 levels are associated with an increased infection risk, we do not conclude that additional vaccine doses increase infection risk. In addition, while our study provides preliminary evidence of an association between IgG4 induction and the risk of breakthrough infection, further research with larger sample sizes and other populations may be required to determine if these findings are generalizable.

These findings may have important implications for future vaccination strategies against SARS-CoV-2. The potential for mRNA vaccines to induce IgG4 and IgG2 responses may necessitate re-evaluation of vaccine formulations or booster schedules. Furthermore, these insights extend beyond COVID-19 and could inform the development of mRNA-based vaccines for other pathogens.

Funding

This work was supported by the European Union under grant agreement no. 101046314 (END-VOC) and by the Fundació Privada Daniel Bravo Andreu. C.M.P. was supported by the 100046TC21_2022 INV-1 00046 grant funded 535 by AGAUR/European Union NextGenerationEU/PRTR. G.M. was

supported by RYC 2020-029886-I/AEI/10.13039/501100011033, co-funded by European Social Fund (ESF). We acknowledge support from the grant CEX2023-0001290-S funded by MCIN/AEI/ 10.13039/501100011033, and support from the Generalitat de Catalunya through the CERCA Program. J.G.B. is funded by an Investigator Grant of the National Health and Medical Research Council of Australia. The funders had no role in study design, data collection and analysis, the decision to publish, or the preparation of the manuscript.

Acknowledgments

We thank the participation of HCW who are committed to this study and were key personnel facing the COVID-19 pandemic. We are grateful to Eduard Velasco at IDIAP JG for the administrative support and the nursing team for their assistance with sample collection. We thank Marianna Spatola at IDIBAPS for facilitating reagents and protocols. We also thank Alfons Jiménez, Diana Barrios, Mar Canyelles, Rocío Rubio, Inocencia Cuamba, Selena Alonso and Laura Puyol, at ISGlobal, and Edwards Pradenas and Benjamin Trinité, at IRSI Caixa, for laboratory support; Pau Serra and Daniel Parras (IDIBAPS) for antigen procurement.

Author contributions

G.M. A.R-C., J.V-A, and C.D. designed the cohort study. A.R-M., J.V-A. and A.R-C. recruited participants, collected data and obtained samples. S.R. and M.V. developed the assays, processed the samples, performed the antibody binding assays and data preprocessing. J.B. performed the antibody neutralization analyses. R.A. contributed to design and the critical interpretation of the results. L.M-A., J.G.B., D.H.O. and P.S. contributed with key reagents and expertise. C.M.P. analyzed the data. G.M., R.A. and C.D. supervised the antibody assays and data analyses. C.M.P., G.M. and C.D. wrote the first draft of the manuscript. All authors reviewed and approved the final version as submitted to the journal.

Conflicts of interest

P. Santamaria is founder, scientific officer and stock holder of Parvus Therapeutics and receives funding from the company. He also has a consulting agreement with Sanofi. The other authors declare no competing interests.

Data and code availability

De-identified data will be deposited at [CORA repository] as [TBD]. The raw identifying data are protected and are not available due to data privacy laws. All original code will be deposited at [CORA repository] and be available at [DOI TBD].

References

1. Irrgang P, Gerling J, Kocher K, et al. Class switch toward noninflammatory, spike-specific IgG4 antibodies after repeated SARS-CoV-2 mRNA vaccination. *Sci Immunol*. 2023;8(79).

2. Lasrado N, Collier ARY, Miller J, et al. Waning immunity and IgG4 responses following bivalent mRNA boosting. *Sci Adv*. 2024;10(8):9945.

3. Buhre JS, Pongracz T, Künsting I, et al. mRNA vaccines against SARS-CoV-2 induce comparably low long-term IgG Fc galactosylation and sialylation levels but increasing long-term IgG4 responses compared to an adenovirus-based vaccine. *Front Immunol*. 2023;13.

4. Marchese AM, Fries L, Beyhaghi H, et al. Mechanisms and Implications of IgG4 Responses to SARS-CoV-2 and Other Repeatedly Administered Vaccines. *Journal of Infection*. 2024;0(0):106317.

5. Akhtar M, Islam MR, Khaton F, et al. Appearance of tolerance-induction and non-inflammatory SARS-CoV-2 spike-specific IgG4 antibodies after COVID-19 booster vaccinations. *Front Immunol*. 2023;14.

6. Gelderloos AT, Verheul MK, Middelhof I, et al. Repeated COVID-19 mRNA vaccination results in IgG4 class switching and decreased NK cell activation by S1-specific antibodies in older adults. *Immunity & Ageing 2024 21:1*. 2024;21(1):1-14.

7. Kalkeri R, Zhu M, Cloney-Clark S, et al. Altered IgG4 antibody response to repeated mRNA versus recombinant protein SARS-CoV-2 vaccines. *Journal of Infection*. 2024;88(3).

8. Klingler J, Kowdle S, Bandres JC, et al. Heterologous Ad26/Ad5 adenovirus-vectored vaccines elicited SARS-CoV-2-specific antibody responses with potent Fc activities. *Front Immunol*. 2024;15.

9. Yoshimura M, Sakamoto A, Ozuru R, et al. The appearance of anti-spike receptor binding domain immunoglobulin G4 responses after repetitive immunization with messenger RNA-based COVID-19 vaccines. *International Journal of Infectious Diseases*. 2024;139:1-5.

10. Kiszel P, Sík P, Miklós J, et al. Class switch towards spike protein-specific IgG4 antibodies after SARS-CoV-2 mRNA vaccination depends on prior infection history. *Sci Rep*. 2023;13(1).

11. Chen W, Zhang L, Li J, et al. The kinetics of IgG subclasses and contributions to neutralizing activity against SARS-CoV-2 wild-type strain and variants in healthy adults immunized with inactivated vaccine. *Immunology*. 2022;167(2):221-232.

12. Vidarsson G, Dekkers G, Rispens T. IgG Subclasses and Allotypes: From Structure to Effector Functions. *Front Immunol*. 2014;5(OCT).

13. Bournazos S, Gupta A, Ravetch J V. The role of IgG Fc receptors in antibody-dependent enhancement. *Nature Reviews Immunology 2020 20:10*. 2020;20(10):633-643.

14. Rispens T, Huijbers MG. The unique properties of IgG4 and its roles in health and disease. *Nature Reviews Immunology 2023 23:11*. 2023;23(11):763-778.

15. Perez CM, Ramirez-Morros A, Jimenez A, et al. Determinants of Antibody Levels and Protection against Omicron BQ.1/XBB Breakthrough Infection. *medRxiv*. Published online October 11, 2024:2024.10.11.24315296.

16. Dobaño C, Vidal M, Santano R, et al. Highly sensitive and specific multiplex antibody assays to quantify immunoglobulins M, A, and G against SARS-CoV-2 antigens. *J Clin Microbiol*. 2021;59(2).

17. Kaplonek P, Fischinger S, Cizmeci D, et al. mRNA-1273 vaccine-induced antibodies maintain Fc effector functions across SARS-CoV-2 variants of concern. *Immunity*. 2022;55(2):355-365.e4.

18. Pradenas E, Trinité B, Urrea V, et al. Stable neutralizing antibody levels 6 months after mild and severe COVID-19 episodes. *Med (N Y)*. 2021;2(3):313-320.e4.

19. Selva KJ, van de Sandt CE, Lemke MM, et al. Systems serology detects functionally distinct coronavirus antibody features in children and elderly. *Nature Communications 2021 12:1*. 2021;12(1):1-14.

20. Yates JL, Ehrbar DJ, Hunt DT, et al. Serological analysis reveals an imbalanced IgG subclass composition associated with COVID-19 disease severity. *Cell Rep Med*. 2021;2(7):100329.

21. Aalberse RC, Schuurman J. IgG4 breaking the rules. *Immunology*. 2002;105(1):9.

22. Krauson AJ, Casimero FVC, Siddiquee Z, Stone JR. Duration of SARS-CoV-2 mRNA vaccine persistence and factors associated with cardiac involvement in recently vaccinated patients. *npj Vaccines 2023 8:1*. 2023;8(1):1-8.

23. Röltgen K, Nielsen SCA, Silva O, et al. Immune imprinting, breadth of variant recognition, and germinal center response in human SARS-CoV-2 infection and vaccination. *Cell*. 2022;185(6):1025-1040.e14.

24. Sanchez L, Vidal M, Jairoce C, et al. Antibody responses to the RTS,S/AS01E vaccine and Plasmodium falciparum antigens after a booster dose within the phase 3 trial in Mozambique. *NPJ Vaccines*. 2020;5(1).

25. Chung AW, Ghebremichael M, Robinson H, et al. Polyfunctional Fc-effector profiles mediated by IgG subclass selection distinguish RV144 and VAX003 vaccines. *Sci Transl Med*. 2014;6(228).

26. Hendrikx LH, Schure RM, Öztürk K, et al. Different IgG-subclass distributions after whole-cell and acellular pertussis infant primary vaccinations in healthy and pertussis infected children. *Vaccine*. 2011;29(40):6874-6880.

27. Ubillos I, Ayestaran A, Nhabomba AJ, et al. Baseline exposure, antibody subclass, and hepatitis B response differentially affect malaria protective immunity following RTS,S/AS01E vaccination in African children. *BMC Med*. 2018;16(1).

28. Chaudhury S, Regules JA, Darko CA, et al. Delayed fractional dose regimen of the RTS,S/AS01 malaria vaccine candidate enhances an IgG4 response that inhibits serum opsonophagocytosis. *Sci Rep*. 2017;7(1).

29. Portilho AI, Silva VO, Da Costa HHM, et al. An unexpected IgE anti-receptor binding domain response following natural infection and different types of SARS-CoV-2 vaccines. *Scientific Reports 2024 14:1*. 2024;14(1):1-12.

30. Hartley GE, Fryer HA, Gill PA, et al. Homologous but not heterologous COVID-19 vaccine booster elicits IgG4+ B-cells and enhanced Omicron subvariant binding. *NPJ Vaccines*. 2024;9(1).

31. Reinig S, Kuo C, Wu CC, Huang SY, Yu JS, Shih SR. Specific long-term changes in anti-SARS-CoV-2 IgG modifications and antibody functions in mRNA, adenovector, and protein subunit vaccines. *J Med Virol*. 2024;96(7):e29793.

32. Liu Z, Cai L, Xing M, et al. Evaluation of antibody responses in healthy individuals receiving SARS-CoV-2 inactivated vaccines. *Biosaf Health*. 2024;6(3):153-164.

33. Chen W, Zhang L, Li J, et al. The kinetics of IgG subclasses and contributions to neutralizing activity against SARS-CoV-2 wild-type strain and variants in healthy adults immunized with inactivated vaccine. *Immunology*. 2022;167(2):221-232.

34. Ackerman ME, Mikhailova A, Brown EP, et al. Polyfunctional HIV-Specific Antibody Responses Are Associated with Spontaneous HIV Control. *PLoS Pathog*. 2016;12(1).

Figures

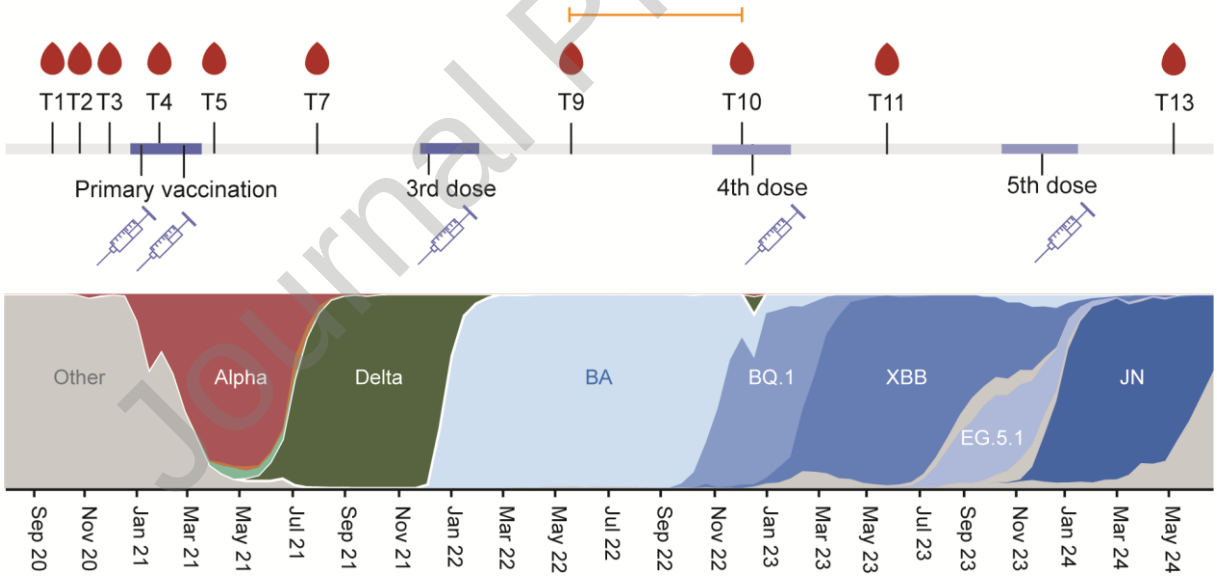

Figure 1. CCC study sample collection and vaccination timepoints, and main SARS-CoV-2 lineages circulating in Spain over the study period. The orange line represents the follow-up period (6 months) in the correlates of protection analysis. All participants received a 3rd dose by T9, while only a subset received a 4th or 5th dose by T13. T: Timepoint.

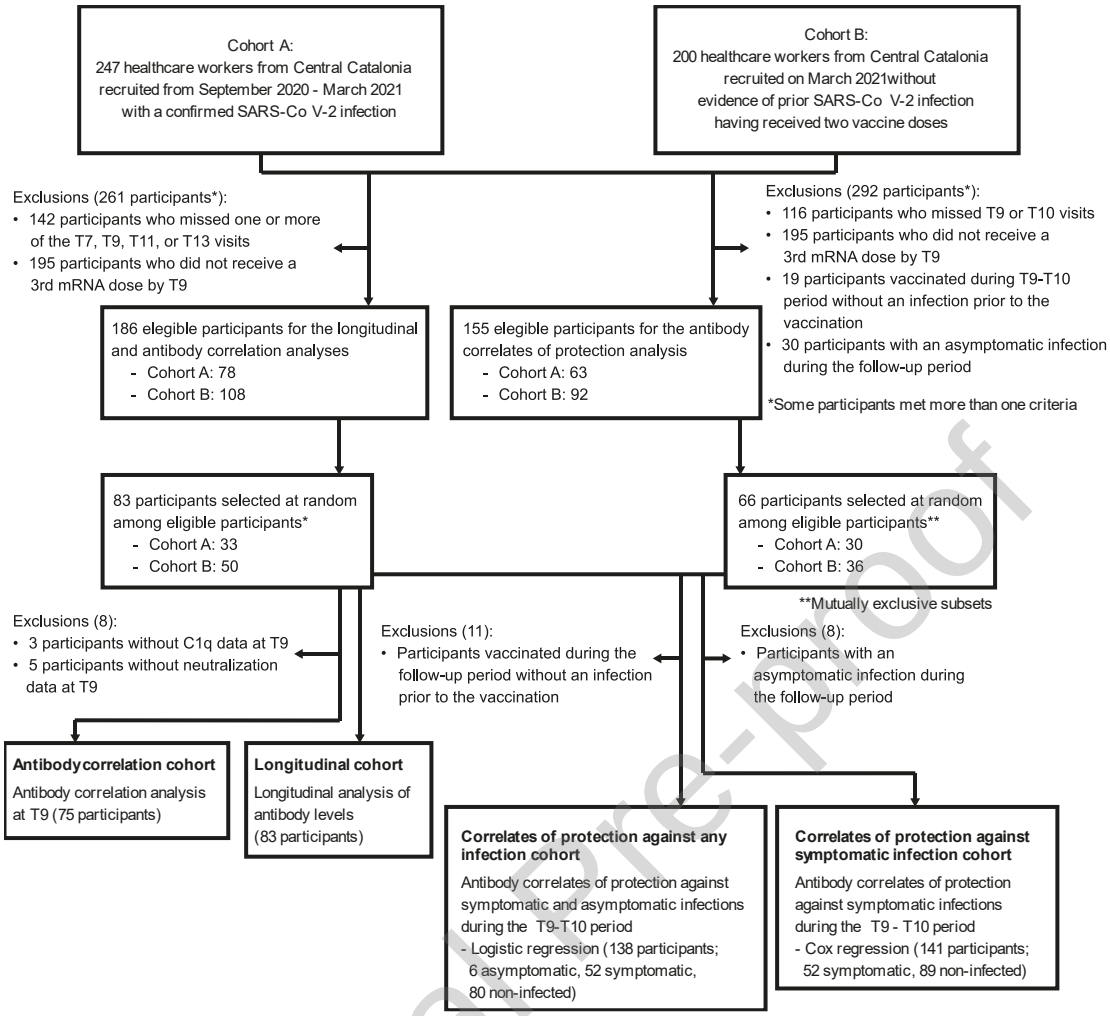

Figure 2. Study profile. This flow diagram outlines the inclusion and exclusion criteria for participants in this study according to the two main analyses (kinetics and correlates of protection) and displays sample sizes at each step.

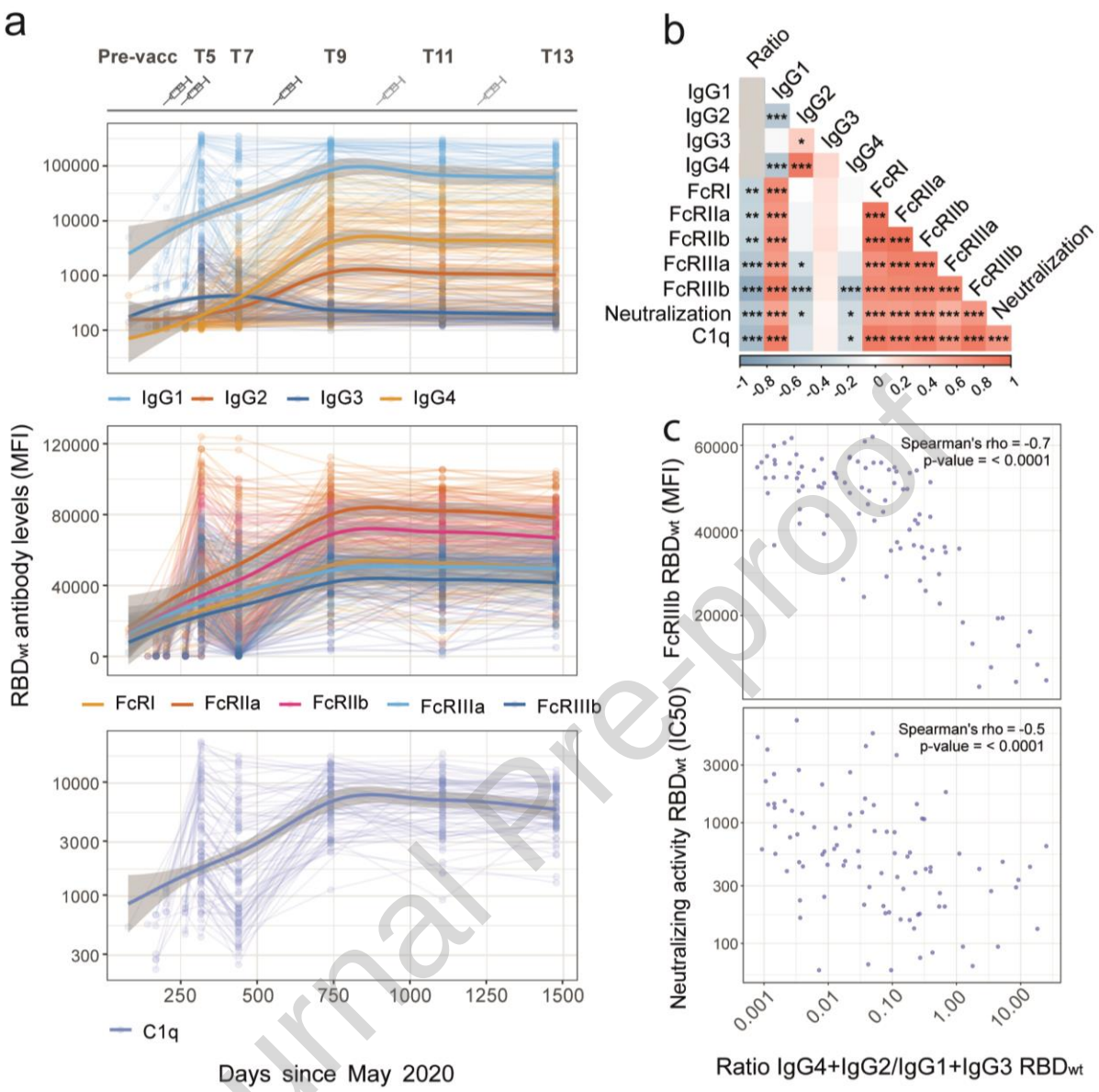

Figure 3. Longitudinal kinetics of IgG subclasses, Fcγ receptor and C1q-binding antibodies in response to COVID-19 mRNA vaccination, and correlations between non-cytophilic to cytophilic antibodies ratio, FcγR binding and neutralizing activity four months after the first booster dose. a. Longitudinal dynamics of antibody levels against the wild type (WT) receptor-binding domain (RBD) of SARS-CoV-2 by IgG subclasses (IgG1, IgG2, IgG3, and IgG4), Fcγ receptor binding (FcγRI, FcγRIIa, FcγRIIb, FcγRIIIa, and FcγRIIIb), and C1q binding (n=83). Positivity threshold is 1199 MFI for C1q, 681 MFI for IgG1, 149 MFI for IgG2, 191 MFI for IgG3, and 127 MFI for IgG4. Similar results were obtained for other spike and RBD variant antigens (**Suppl Fig. 1, 2**). Vaccination timepoints are indicated at the top, with black marks representing doses received by all participants and grey marks indicating doses received by a subset of the participants (27 received a fourth dose between T9 and T11, and 7 received a fifth dose between T11 and T13). A locally estimated scatterplot smoothing (LOESS) curve was fitted to each antibody measure to visualize trends over time. Shaded areas indicate 95% confidence intervals. Pre-vaccination samples were obtained July 2020–February 2021, and post-vaccination on timepoints (T)5 March–April 2021, T7 July 2021, T9 May 2022, T11 May–June 2023, and T13 May 2024. MFI values for IgG subclasses and Fc functions have not been normalized; therefore, comparisons between absolute levels across

different subclasses or Fcγ-binding antibodies should not be made. **b**. Correlogram showing the Spearman correlations between the ratio of non-cytophilic (IgG4+IgG2) to cytophilic (IgG1+IgG3) antibodies, C1q and FcγR-binding levels to RBD WT, and neutralizing activity against WT SARS-CoV-2. Positive correlations are indicated in red, while negative correlations are shown in blue. Statistically significant correlations are marked with asterisks (n = 75). **c.** Correlation between the ratio of non-cytophilic to cytophilic antibodies binding to RBD WT and both FcγRIIIb binding levels and neutralizing activity against WT SARS-CoV-2 (n = 75). Spearman's correlation coefficients (rho) and p-values adjusted for multiple comparisons by Benjamini-Hochberg are indicated in the plots.

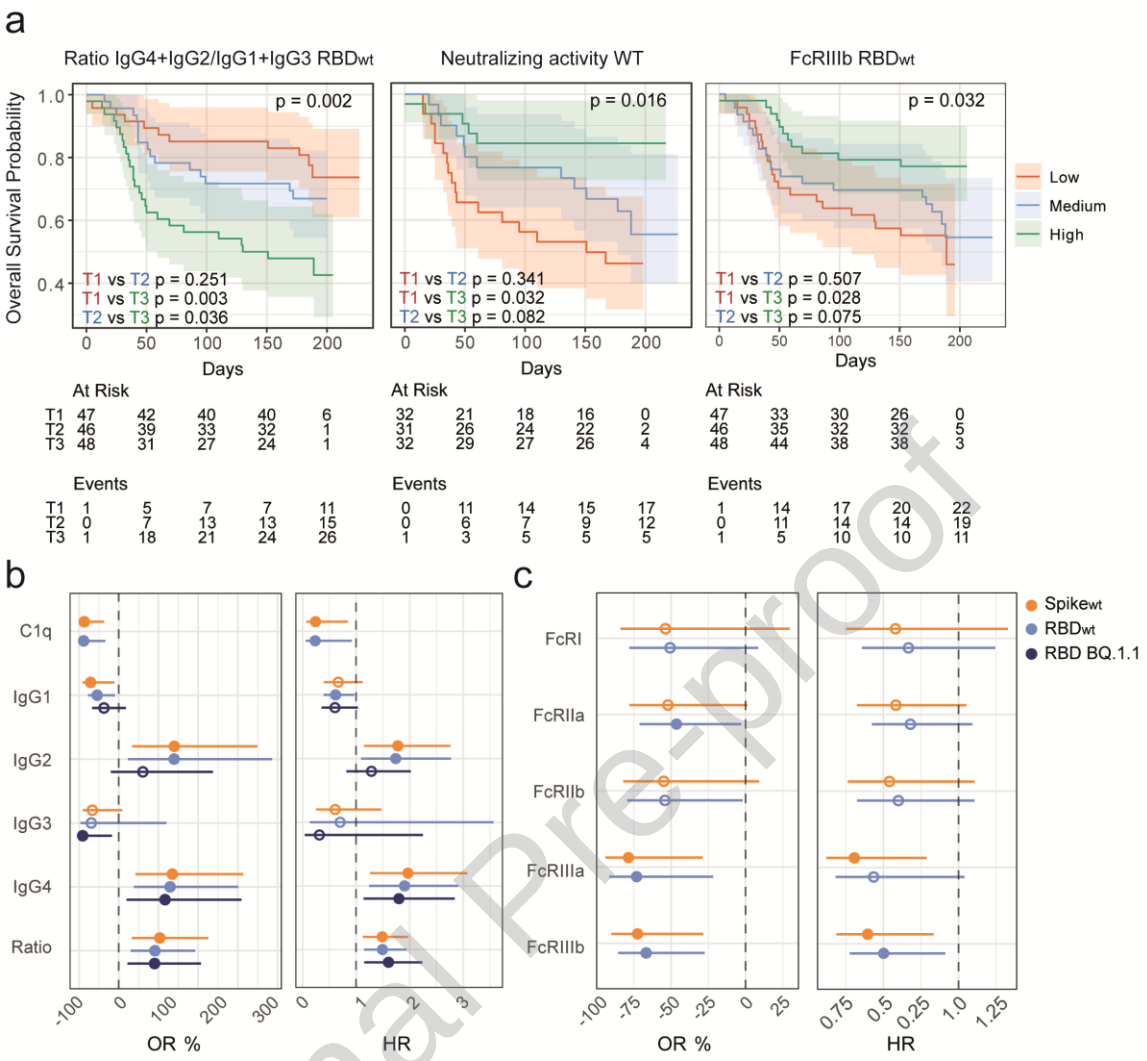

Figure 4. Association of IgG subclass levels, plasma neutralizing activity and Fc receptor-binding antibodies four months after the first booster dose with protection against COVID-19 and SARS-CoV-2 infections in vaccinated individuals. a. Kaplan–Meier survival curves of risk of symptomatic breakthrough infection by tertiles of the ratio of non-cytophilic (IgG2+IgG4) to cytophilic (IgG1+IgG3) antibodies, neutralizing activity, and FcRIIIb-binding antibodies, to wild type (WT) receptor binding domain (RBD) (n = 141). Analysis of antibodies to other spike antigens, variants, and FcγR/C1q binding, yielded similar results (Suppl Fig. 6). Red corresponds to the lowest antibody level tertile, whereas green denotes the highest tertile. Shaded areas represent the 95% confidence intervals (CI). Kaplan–Meier curves were compared using the log-rank test. Benjamini-Hochberg-adjusted p-values for pairwise comparisons are shown on each plot. **b.** Forest plot of multivariable logistic regression models (n = 138) and Cox proportional hazard models (n=141) for C1q-binding antibodies, IgG subclasses, and ratio of non-cytophilic to cytophilic antibodies to WT spike and RBD, and to RBD from the Omicron BQ.1.1 variant. Odds Ratio (OR) represents the percent change in the odds of infection associated with a 10-fold increase in median fluorescence intensity (MFI). Hazard ratio (HR) represents the change in the relative hazard associated with a 10-fold increase in MFI. Models were adjusted by age, comorbidities, number of infections, sex, smoking, time since last exposure, and first exposure type. Filled dots indicate p <0.05, while unfilled dots indicate non-significant p-values. OR and CI values have been transformed to a percentage for an easier interpretation. **c.** Forest plot of multivariable logistic

regression models (n = 138) and Cox proportional hazard models (n = 141) for Fc receptor-binding antibodies to WT spike and RBD. OR represents the percent change in the odds of infection associated with one SD increase in MFI. HR represents the change in the relative hazard associated with one SD increase in MFI.

Table 1. Characteristics of study participants included in the correlates of protection against symptomatic infections cohort, correlates of protection against any (symptomatic or asymptomatic) infection cohort, longitudinal cohort, and antibody correlation cohort at T9.

		N or Median (IQR)			
		CoP symptomatic infections cohort	CoP any infections cohort	Longitudinal cohort	Antibody correlation cohort
Age		49 (15 IQR)	49 (16 IQR)	52 (14.5 IQR)	53 (14.5 IQR)
Sex	Female	119	117	70	64
	Male	22	21	13	11
Comorbidities*	Yes	95	93	54	48
	No	46	45	29	27
Smoking	Yes	52	53	28	25
	No	89	85	55	50
Vaccine doses	3	141	138	83	75
	0	47	46	31	26
Number of previous infections	1	69	63	39	37
	2	23	22	11	10
	3	2	1	2	2
First dose type	Pfizer	140	137	82	74
	Moderna	1	1	1	1
Second dose type	Pfizer	140	137	82	74
	Moderna	1	1	1	1
Third dose type	Pfizer	53	53	36	32
	Moderna	83	83	47	43
	NA	2	2	0	0
Time since last vaccination		152 (17 IQR)	144 (39 IQR)	150 (41.5 IQR)	150 (41.5 IQR)

* Includes COPD, asthma, cancer, diabetes, obesity, depression, hypothyroidism, dyslipidemia, chronic kidney disease, hypertension, and cardiac, neurological, digestive, autoimmune diseases.

Table 2. Median fold changes (FC) in antibody levels with interquartile ranges (IQR) between consecutive timepoints (n = 83). Primary vaccination occurred before timepoint (T)5, with the first booster administered between T7 and T9. A subset of participants (n = 27) received a fourth dose between T9 and T11, and another subset (n = 7) received a fifth dose between T11 and T13.

	Pre-primary vacc -T5		T5 - T7		T7 - T9		T9-T11		T11-T13	
Immune measure	**FC [IQR]**	**P-value***	**FC [IQR]**	**P-value***	**FC [IQR]**	**P-value***	**FC [IQR]**	**P-value***	**FC [IQR]**	**P-value***
FcRI	9.99 [18.42]	< 0.0001	0.57 [0.31]	< 0.0001	2.74 [2.64]	< 0.0001	0.96 [0.21]	0.32	0.95 [0.13]	0.02
FcRIIIa	25.28 [271.56]	< 0.0001	0.48 [0.46]	< 0.0001	2.70 [4.18]	< 0.0001	0.98 [0.18]	0.39	1 [0.13]	0.28
FcRIIIb	129.34 [297.47]	< 0.0001	0.34 [0.6]	< 0.0001	3.50 [17.46]	< 0.0001	1 [0.27]	0.21	0.99 [0.22]	0.15
FcRIIa	48.63 [408.13]	< 0.0001	0.46 [0.46]	< 0.0001	3.22 [4.6]	< 0.0001	0.98 [0.22]	0.75	0.97 [0.14]	0.88
FcRIIb	45.97 [452.21]	< 0.0001	0.46 [0.6]	< 0.0001	3.17 [6.09]	< 0.0001	0.97 [0.19]	0.64	0.97 [0.09]	0.85
IgG1	188.3 [251.9]	< 0.0001	0.20 [0.42]	< 0.0001	8.00 [40.3]	< 0.0001	0.87 [0.71]	0.08	0.85 [0.71]	0.09
IgG2	1.39 [0.52]	< 0.0001	1.02 [0.3]	0.35	5.39 [9.56]	< 0.0001	0.9 [0.38]	0.19	0.91 [0.29]	0.03
IgG3	1.79 [0.87]	< 0.0001	0.44 [0.31]	< 0.0001	0.88 [0.61]	0.02	0.89 [0.25]	< 0.01	0.93 [0.15]	0.03
IgG4	1.05 [0.14]	0.04	2.42 [3.51]	< 0.0001	10.85 [19.6]	< 0.0001	0.91 [0.67]	0.2	0.88 [0.23]	0.07
C1q	15.79 [7.94]	< 0.0001	0.50 [0.27]	< 0.0001	4.57 [6.74]	< 0.0001	0.94 [0.57]	0.11	0.92 [0.4]	0.08

* Differences in antibody levels were assessed using the Wilcoxon Signed-Rank test and adjusted for multiple comparisons via the Benjamini-Hochberg method.

Declaration of Competing Interest

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

P. Santamaria is founder, scientific officer and stock holder of Parvus Therapeutics and receives funding from the company. He also has a consulting agreement with Sanofi. The other authors declare no competing interests.

Highlights

- IgG4 and IgG2 levels increase markedly after the third mRNA dose against SARS-CoV-2.
- Elevated IgG4 levels after booster vaccination associate with an increased risk of infections.
- Increased non-cytophilic to cytophilic antibody ratio correlates with reduced functionality.