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ORIGINAL ARTICLE

Suppressed IgG4 class switching in dupilumab- and TNF inhibitor-treated patients after mRNA vaccination

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Abstract

Background: The noninflammatory immunoglobulin G4 (IgG4) is linked to tolerance and is unique to humans. Although poorly understood, prolonged antigenic stimulation and IL-4-signaling along the T helper 2-axis may be instrumental in IgG4 class switching. Recently, repeated SARS-CoV-2 mRNA vaccination has been linked to IgG4 skewing. Although widely used immunosuppressive drugs have been shown to only moderately affect humoral responses to SARS-CoV-2 mRNA vaccination, the effect on IgG4 switching has not been investigated.

Methods: Here we study the impact of such immunosuppressive drugs, including the IL-4 receptor-blocking antibody dupilumab, on IgG4 skewing upon repeated SARS-CoV-2 mRNA vaccination. Receptor-binding domain (RBD) specific antibody responses were longitudinally measured in 600 individuals, including patients with immune-mediated inflammatory diseases treated with a TNF inhibitor (TNFi) and/or methotrexate (MTX), dupilumab, and healthy/untreated controls, after repeated mRNA vaccination.

Results: We observed a substantial increase in the proportion of RBD-specific IgG4 antibodies (median 21%) in healthy/untreated controls after third vaccination. This IgG4 skewing was profoundly reduced in dupilumab-treated patients (<1%). Unexpectedly, an equally strong suppression of IgG4 skewing was observed in TNFi-treated patients (<1%), whereas MTX caused a modest reduction (7%). RBD-specific total IgG levels

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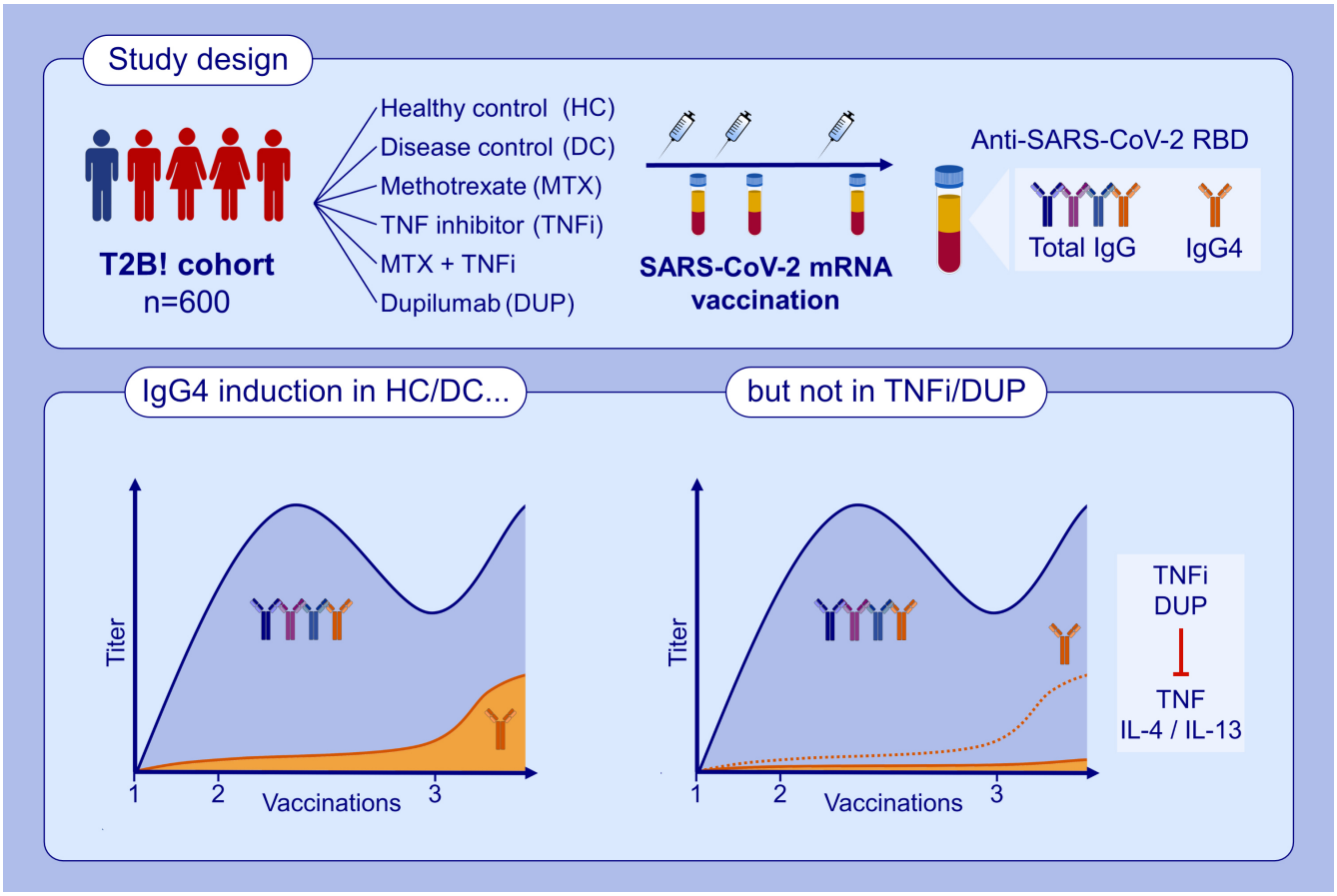
were hardly affected by these immunosuppressive drugs. Minimal skewing was observed, when primary vaccination was adenoviral vector-based.

Conclusions: Our results imply a critical role for IL-4/IL-13 as well as TNF in vivo IgG4 class switching. These novel findings advance our understanding of IgG4 class switch dynamics, and may benefit humoral tolerance induction strategies, treatment of IgG4 pathologies and mRNA vaccine optimization.

KEYWORDS
dupilumab, IgG4 class switching, mRNA vaccination, TNFi, tolerance



GRAPHICAL ABSTRACT

- This study assessed total IgG and IgG4 anti-RBD responses after SARS-CoV-2 mRNA vaccination in a longitudinal cohort comprising healthy controls (HC), disease controls (DC), patients treated with methotrexate (MTX), TNF inhibitors (TNFis), the combination thereof and dupilumab.
- We found significant IgG4 skewing in HC and DC after repeated vaccination, and to a lesser extent in patients treated with MTX.
- IgG4 skewing was virtually absent in patients treated with dupilumab or TNFis, illustrating major roles for IL-4/IL-13 and TNF in the overall process of class switching to IgG4.

1 | INTRODUCTION

Immunoglobulin G4 (IgG4) is a peculiar antibody subclass exclusively found in humans. It has a distinct noninflammatory character, with low affinity to most FcγRs and C1q, and therefore a limited potential for antibody dependent cellular cytotoxicity (ADCC) and complement-mediated effector functions.[1,2] IgG4 is also uniquely able to undergo Fab-arm exchange, resulting in a bispecific antibody that is functionally monovalent and unable to form large immune complexes, reducing its inflammatory potential even more.[3–6] However, IgG4 is generally associated with high levels of somatic hypermutation (SHM) and high affinity antigen binding, contributing to efficient neutralization. Due to this combination of weak effector functions yet high neutralization capacity, IgG4 has been referred to as a blocking antibody. As

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such, IgG4 plays a diverse role in various pathological settings. In the context of specific immunotherapy, IgG4 may contribute to tolerance by inhibiting allergen-specific IgE. On the other hand, tumor-specific IgG4 has been implicated in immune evasion in melanoma,[7] whereas in autoimmunity, IgG4 autoantibodies can be pathogenic solely on the basis of blocking their target, as is the case for anti-desmoglein antibodies in pemphigus vulgaris.[8–11]

A crucial question is what is driving the switch towards noninflammatory IgG4. Although key drivers of IgG4 class switching remain to be elucidated, prolonged antigenic stimulation and signaling along the T helper 2 (Th2) axis have been shown to play a role.[12–14] Class switching to IgG4 has recently been described during repeated SARS-CoV-2 mRNA vaccination, resulting in a substantial portion of the anti-spike antibody response to consist of the IgG4 subclass.[15–17] The robust germinal centers with long lasting spike antigen found after repeated SARS-CoV-2 mRNA vaccination could potentially contribute to IgG4 skewing, although the exact drivers are still unclear.[18,19] While IgG4 class switching is a relatively uncommon event upon vaccination, there are other examples of vaccine-induced antigen-specific IgG4 induction. The nature of the primary antigen challenge appears crucial in these cases. For instance, during HIV vaccination trials IgG4 switching was exclusively seen in response to bivalent recombinant protein-based vaccines (VAX003/VAX004 trails), but not after viral vector-based primary immunization.[20,21] Furthermore, in case of pertussis vaccination, the acellular vaccine induced a stronger IgG4 switch in comparison to a whole cell vaccine variant.[22,23] On the other hand, repeated tetanus toxoid immunization to produce hyperimmune anti-tetanus serum results in an IgG1-dominated response,[24] illustrating that repeated vaccination in itself does not necessarily induce IgG4 class switching. Mechanistically, IgG4 switching has been associated with Th2 responses *in vivo*, and IL-4 has been identified *in vitro* cultures as an important cytokine for IgG switching in general and IgG4 in particular.[25–31] Other cytokines that have been associated with (selective) IgG4 induction include IL-10 and IL-13, but details as to their involvement remain unclear.[2]

A potential concern with vaccinations is the response in patients treated with immunosuppressive medications. These drugs target different elements of the immune system and may interfere with vaccination strategies to activate the immune system. We and others have previously shown that the impact thereof varies greatly. Widely used drugs, such as methotrexate (MTX) and TNF inhibitors (TNFis) appear to have an overall limited impact on the humoral response to SARS-CoV-2 mRNA vaccines, yet show slightly diminished antibody titers.[32–35] Whether or not such immunosuppressive drugs may further enhance or prevent switching to the noninflammatory IgG4 isotype is unknown.

In this study, we investigated the impact of several widely used immunosuppressive drugs on IgG4 switching of the anti-spike antibody response upon repeated mRNA vaccination in a large cohort of individuals, including patients treated with the IL-4R blocking antibody dupilumab, as well as TNFi, MTX, or a combination of TNFi and MTX. Studying the dynamics of a *de novo* IgG4 response upon specific therapeutic inhibition of certain cytokines may provide valuable *in vivo* mechanistic insight in IgG4 class switching, which is not possible in laboratory animals, since these lack an IgG4 equivalent.

2 | MATERIALS AND METHODS

2.1 | Study design

This study, part of the previously described Target-2-B! Immunity against SARS-CoV-2 vaccination cohort[32] included immune mediated inflammatory disease (IMID) patients treated with MTX, TNFi, the combination thereof, or dupilumab. Patients had no history of oncological or hematological disorders. Control groups consisting of IMID patients that were not treated with systemic immunosuppressants (disease controls, DC) and healthy controls (HC). Participants received two homologous doses of BNT162b2, mRNA-1273 or ChAdOx1 nCoV-19, followed by a booster dose of BNT162b2 or mRNA-1273. Serum samples were collected 28 days after each vaccination and before the first and third vaccination by at-home fingerprick, as described previously.[32] Participants who experienced a SARS-CoV-2 infection prior to or during the initial two-dose vaccination regimen, monitored as previously described using anti-RBD and anti-N serology (see below) and positive PCR results reported to the research team,[32] were excluded from this sub-study. Breakthrough infections were found in a total of 17 participants: two HC, three DC, one patient treated with MTX, one patient treated with dupilumab, eight patients treated with TNFi (of whom three received ChAdOx1 nCoV-19 for their initial vaccinations) and two patients treated with MTX and TNFi (Figures S2 and S4). This study was approved by the medical ethical committee of the Amsterdam UMC (2020.194; trial registry NL74974.018.20 and EudraCT 2021-001102-30). All participants provided written informed consent.

2.2 | Anti-RBD and -N total antibody ELISAs

To monitor (breakthrough) infections in the cohort through qualitative detection of total antibodies (pan-isotype) directed against the receptor binding domain (RBD) of the spike (S) protein and the nucleocapsid (N) protein of SARS-CoV-2 (Wuhan-Hu-1) in serum samples, we performed previously described in-house developed bridging enzyme-linked immunosorbent assays (ELISAs).[36] Anti-RBD was tested in samples taken before vaccination and anti-N in samples from all subsequent time points.

2.3 | Anti-RBD IgG ELISAs

To quantify total IgG directed against the RBD of SARS-CoV-2 (Wuhan-Hu-1) in serum samples, we performed a previously described in-house developed direct ELISA.[36,37] In short, this

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assay was calibrated using a pooled plasma standard obtained from convalescent healthy donors in May 2020, which was set at 100 arbitrary units (AU)/mL. The lower limit of quantification for samples tested at 1:1200 dilution was 1 AU/mL, with a > 99% specificity cutoff determined at 4 AU/mL.[37] This format was further adapted into an IgG4-specific ELISA. 96-well half-area microplates (Corning) with a working volume of 50 µL were coated overnight at 4°C with 1 µg/mL RBD in phosphate buffered saline (PBS; Fresenius Kabi). Plates were washed five times with PBS + 0.02% v/v Tween-20 (Merck, Germany) using an ELx405 ELISA washer (Biotek Instruments). Serum samples were diluted 1:200 in PBS + 0.1% v/v Tween-20 + 2 g/L gelatin (Merck, Germany; PTG), added to the wells and incubated for 1 h at RT. After washing, 0.5 µg/mL anti-human IgG4-HRP (MH164-4-HRP, Sanquin) in PTG was added and incubated for 30 min at RT. After one more washing step, 1-step Ultra TMB substrate (Thermo Scientific) diluted with milli-Q water in a ratio of 3:1 was added to wells. Reactions were stopped after approximately 7 min with an additional 50 µL 0.2 M H_2SO_4 and optical density (OD) was measured at 450 and 540 nm with a Synergy 2 microplate reader (BioTek Instruments). As a calibrator, the previously described COVA1-18 anti-RBD clone[38] was engineered with a human IgG4 heavy chain, analogously as described previously for IgG1 and IgG3.[37] The calibrator was twofold serially diluted starting from 0.025 µg/mL with a PTG blank. When tested in this manner in the total IgG assay, the IgG4 monoclonal demonstrated good linearity of dilution and parallelism with the 2020 pooled plasma standard starting from 0.25 AU/mL (1:400; Figure S1A). We thus calculated that 1 AU of 2020 pooled plasma produced similar IgG detection signal to 0.11 µg of IgG4 monoclonal, and IgG4 measurements could be expressed in AU/mL equivalent for comparison to total IgG measurements. Lastly, we determined a lower limit of quantification of 0.1 AU/mL (approx. 11 ng/mL) for samples tested at 1:200 dilution and a >99% specificity cutoff of 0.3 AU/mL (approx. 33 ng/mL) using a panel of negative pre-2020 samples (Figure S1B). The anti-RBD IgG4 assay is more sensitive in comparison to anti-RBD total IgG, due to the lower background signals in line with the generally much lower total IgG4 levels in sera compared to total IgG.

2.4 | Statistical analysis

Ratios of anti-RBD IgG4 titers divided by anti-RBD total IgG after third vaccination (V3) were analyzed using the Kruskal–Wallis test and the Conover–Iman post hoc multiple comparisons test with Benjamini–Hochberg (FDR) correction. A lower limit of 0.005 was imputed for ratio values, as lower ratios only resulted from IgG4 titer values below seroconversion cutoff. Analysis and visualization was performed using R version 4.1.2[39] with packages "tidyverse" version 1.3.1,[40] "conover. test" version 1.1.5, "scales" version 1.2.1, "ggpubr" version 0.4.0 and "patchwork" version 1.1.2.

3 | RESULTS

3.1 | Participant characteristics

Samples from 600 participants of the ongoing Target-2-B! Immunity against SARS-CoV-2 vaccination cohort were retrieved for this study (for epidemiologic and demographic characteristics see Table 1). The mean age of participants was 53.0 years (SD 14.2) and 62.0% were female. Vaccinations were given between February 2021 and May 2022; the median interval between first and second doses was 36 days (IQR 35–42) and 190 days (IQR 176–200) between second and third. Participants were sampled 28 days after each vaccination, and just prior to the third vaccination.

3.2 | Differential induction of anti-RBD IgG4

A robust anti-RBD total IgG response was observed at 28 days after the first and second mRNA vaccination dose. Antibody levels had declined by about an order of magnitude just prior to the third vaccination, and returned to post-second levels 28 days after the third dose in all groups (Figure 1A). Anti-RBD IgG4 levels were very low after the first and second mRNA vaccination dose (Figure 1B,C). However, in HC and DC, the IgG4 antibody response was greatly boosted by the third mRNA vaccination dose (Figure 1B,C), yielding a sharp increase in the proportion of RBD-specific IgG4 (median 21.7% (IQR 6.6%–46.0%) and 14.1% (IQR 2.2%–73.2%), respectively; Figure 2). Conversely, IgG4 levels remained very low in patients treated with dupilumab compared to HC and DC, with a median proportion of less than 1%. TNFi treatment similarly reduced the induction of RBD-specific IgG4, both as single agent, as well as in combination with MTX. Patients treated with MTX monotherapy exhibited a modestly reduced induction of IgG4 skewing after the third dose with a median proportion of 6.8% (IQR 0.8%–34.2%). Total IgG and IgG4 responses of individual participants are shown in Figure S2. We found no significant correlation between the relative contribution of IgG4 to total IgG after third vaccination and participant age or different combinations of BNT162b2 and mRNA-1273 (data not shown). Breakthrough infections after second vaccination had no consistent impact on IgG4 induction. In contrast, induction of IgG4 was virtually absent in all individuals initially vaccinated with two doses of ChAdOx1 nCoV-19, regardless of immunosuppressive treatment or breakthrough infection (Figures S3 and S4). Taken together, we observe a profound suppression of repeated mRNA vaccination-driven RBD-specific IgG4 induction by both the IL-4R blocking antibody dupilumab and TNFi, while initial viral vector-based vaccination did not induce a relevant IgG4 response.

4 | DISCUSSION

Here we longitudinally analyzed IgG4 RBD-specific antibody responses in a large cohort consisting of 600 participants overall. In

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TABLE 1 Demographic and clinical characteristics of study participants.

	Overall (*N* = 600)	Healthy controls (*N* = 107)	Disease controls (*N* = 50)	Patients on MTX (*N* = 125)	Patients on DUP (*N* = 61)	Patients on TNFi (*N* = 193)	Patients on MTX + TNFi (*N* = 64)
Age (years), mean (SD)	53.0 (14.2)	51.0 (10.3)	54.1 (13.8)	59.6 (12.2)	45.6 (13.7)	49.8 (15.2)	58.8 (13.8)
Sex							
Female	372 (62.0%)	69 (64.5%)	28 (56.0%)	93 (74.4%)	30 (49.2%)	108 (56.0%)	44 (68.8%)
Male	228 (38.0%)	38 (35.5%)	22 (44.0%)	32 (25.6%)	31 (50.8%)	85 (44.0%)	20 (31.3%)
Initial vaccines							
BNT162b2 (Pfizer-BioNTech)	348 (58.0%)	47 (43.9%)	22 (44.0%)	63 (50.4%)	57 (93.4%)	119 (61.7%)	40 (62.5%)
mRNA-1273 (Moderna)	178 (29.7%)	58 (54.2%)	16 (32.0%)	38 (30.4%)	3 (4.9%)	48 (24.9%)	15 (23.4%)
ChAdOx1 nCoV-19 (AstraZeneca)	66 (11.0%)	2 (1.9%)	10 (20.0%)	23 (18.4%)	1 (1.6%)	22 (11.4%)	8 (12.5%)
Booster vaccine							
BNT162b2 (Pfizer-BioNTech)	143 (23.8%)	33 (30.8%)	8 (16.0%)	23 (18.4%)	17 (27.9%)	48 (24.9%)	14 (21.9%)
mRNA-1273 (Moderna)	235 (39.2%)	37 (34.6%)	10 (20.0%)	77 (61.6%)	12 (19.7%)	73 (37.8%)	26 (40.6%)
Unspecified mRNA	135 (22.5%)	22 (20.6%)	21 (42.0%)	14 (11.2%)	19 (31.1%)	48 (24.9%)	11 (17.2%)
None	87 (14.5%)	15 (14.0%)	11 (22.0%)	11 (8.8%)	13 (21.3%)	24 (12.4%)	13 (20.3%)
Vaccination interval (days), median (IQR)							
V1–V2	36 (35–42)	37 (35–42)	39 (35–42)	36.0 (35.0–42.0)	35 (35–36)	36 (35–42)	36.0 (35.0–42.0)
V2–V3	190 (176–200)	190 (181–197)	192 (182–205)	186 (118–198)	194 (180–200)	190 (175–199)	193 (187–204)
Rheumatic disorders							
Rheumatoid arthritis	139 (23.2%)	–	1 (2.0%)	69 (55.2%)	–	26 (13.5%)	43 (67.2%)
Spondylarthritis	68 (11.3%)	–	–	13 (10.4%)	–	42 (21.8%)	13 (20.3%)
Systemic lupus erythematosus	4 (0.7%)	–	–	4 (3.2%)	–	–	–
Vasculitis[a]	4 (0.7%)	–	3 (6.0%)	1 (0.8%)	–	–	–
Other rheumatological[b]	4 (0.7%)	–	1 (2.0%)	2 (1.6%)	–	1 (0.5%)	–
Gastro-intestinal disorders							
Crohn's disease	105 (17.5%)	–	5 (10.0%)	1 (0.8%)	–	95 (49.2%)	4 (6.3%)
Ulcerative colitis	62 (10.3%)	–	34 (68.0%)	–	–	28 (14.5%)	–
Other gastro-intestinal[c]	2 (0.3%)	–	1 (2.0%)	–	1 (1.6%)	–	–
Neurological disorders							
Inflammatory neuropathies and myopathies[d]	5 (0.8%)	–	1 (2.0%)	4 (3.2%)	–	–	–
Other neurological[e]	3 (0.5%)	–	2 (4.0%)	1 (0.8%)	–	–	–
Dermatological disorders							
Atopic dermatitis	61 (10.2%)	–	–	3 (2.4%)	58 (95.1%)	–	–
Other dermatological[f]	36 (6.0%)	–	2 (4.0%)	27 (21.6%)	2 (3.3%)	1 (0.5%)	4 (6.3%)

Note: Data are presented as *n* (%) unless noted otherwise.

[a]Including small-vessel, medium-vessel, and large-vessel vasculitis and other forms of vasculitis except giant cell arteritis.

[b]Including Sjögren's syndrome, polymyalgia rheumatica, and juvenile arthritis.

[c]Two patients with autoimmune hepatitis.

[d]Including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy, and inflammatory myositis.

[e]Including myasthenia gravis and multiple sclerosis.

[f]Including psoriasis, vitiligo and others.

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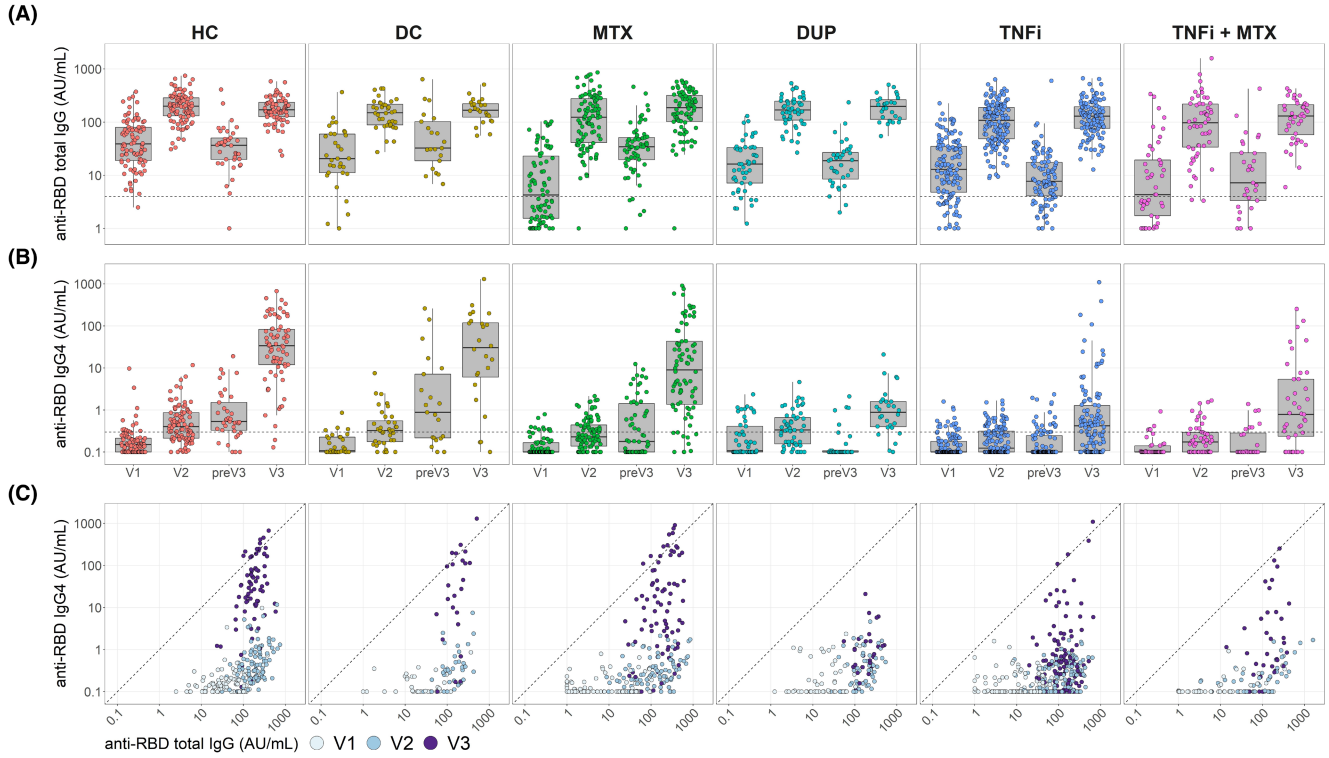

FIGURE 1 Longitudinal RBD-specific total IgG and IgG4 antibody response in healthy controls (HC), disease controls (DC) and treatment groups after mRNA vaccination. Serum samples were collected 28 days after first, second, and third vaccination (V1, V2, and V3) and immediately prior to third (preV3). Anti-RBD titers were assessed by direct ELISA and calculated in arbitrary units (AU) derived from pooled convalescent healthy donor plasma standards collected in early 2020 (total IgG), which was set at 100 AU/mL, or a monoclonal standard (IgG4) expressed in equivalent AU/mL. (A, B) Box plots showing RBD-specific total IgG (A) and IgG4 (B). Central lines in box plots indicate the median, with hinges indicating 25th and 75th percentiles. Dashed lines represent seropositivity cutoffs, 4 AU/mL for total IgG and 0.3 AU/mL for IgG4, determined as the AU value where >99% of pre-pandemic samples were considered negative. (C) Scatter plots showing RBD-specific total IgG and IgG4, as in (A) and (B), respectively. Dashed diagonal lines indicate 1:1 titer ratio.

accordance with previous findings, we report an increase in RBD-specific IgG4 after a third SARS-CoV-2 mRNA vaccination, which was virtually absent after primary adenoviral vector-based vaccination. This IgG4 skewing was profoundly reduced in patients treated with the IL-4R-blocking antibody dupilumab, as well as with several widely used TNFi. Given the role of Th2 responses in IgG4 switching it may not be wholly unexpected that blockade of IL-4 signaling impairs IgG4 induction, although to the best of our knowledge this is the first actual *in vivo* demonstration thereof. On the other hand, the inferred role for TNF signaling as indicated by our results is surprising. These novel findings advance our understanding of IgG4 class switch dynamics, and may benefit humoral tolerance induction strategies, treatment of IgG4 pathologies, and mRNA vaccine optimization.

Dupilumab blocks the IL-4/IL-13 receptor and is used to treat atopic dermatitis. These cytokines are typical for the Th2 axis that has been associated with IgG4 switching.[25–31] Nevertheless, we are not aware of previous studies demonstrating inhibition of *de novo* IgG4 skewing by dupilumab. T follicular helper (Tfh) cells are a major source of IL-4 and persistent Tfh cells have been found after repeated SARS-CoV-2 mRNA vaccination, which could potentially facilitate IgG4 class switching.[41] The suppressed RBD-specific IgG4 titer after third vaccination in the dupilumab-treated group indicates a pivotal role of IL-4 signaling in IgG4 switching, and may present an opportunity for therapeutic intervention in undesired IgG4 responses. Furthermore, these findings may support the rationale behind the consideration of dupilumab as a potential treatment for IgG4-related disease (IgG4-RD), a group of immune-mediated fibrotic diseases that affect several organs and are characterized by high serum IgG4 levels plus increased tissue infiltrating $IgG4^+$ plasma cells.[10] The role that had been attributed to IL-4 together with Th2 cells in IgG4 class switching has led to several case studies treating IgG4-RD patients with dupilumab. However, controversial outcomes in these studies have fueled an ongoing discussion on whether dupilumab treatment is beneficial in IgG4-RD or not.[42] Furthermore, dupilumab did not inhibit the increase of the specific IgG4 response when used as an adjuvant during allergen-specific immunotherapy.[43] It may therefore be possible that IL-4R blockade primarily affects (*de novo*) IgG4 class switching, but not expansion of established IgG4-switched B cell populations. In the context of vaccination, one study reports that correlates of vaccine-induced immunity were not observed for tetanus toxoid vaccination.[44] However, IgG4 skewing is very limited for tetanus toxoid vaccination even upon repeated boosting.[24]

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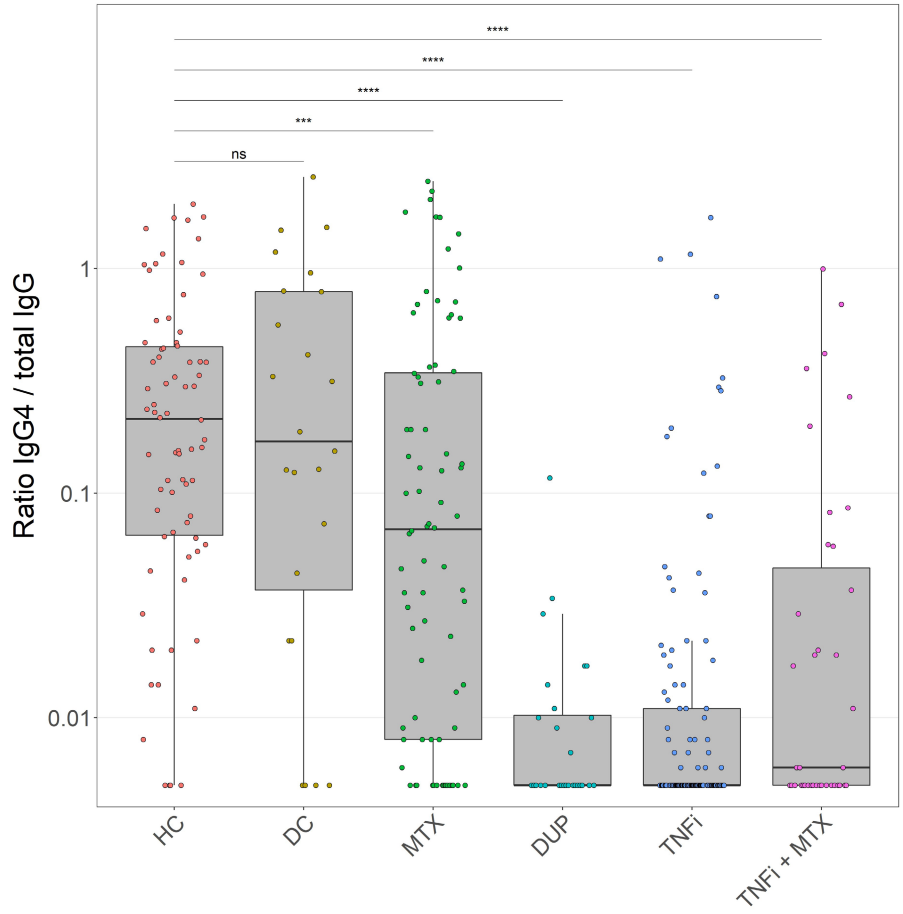

FIGURE 2 Ratio of RBD-specific IgG4 over RBD-specific total IgG in healthy controls (HC), disease controls (DC) and treatment groups after third mRNA vaccination. Box plots showing ratios computed as RBD-specific IgG4 / RBD-specific total IgG in post-third vaccination samples (V3). In all box plots, central lines indicate the median, with hinges indicating 25th and 75th percentiles. Whiskers indicate the furthest data points up to 1.5 * IQR beyond hinges. Comparisons were made using Conover–Iman post hoc multiple comparisons with Benjamini–Hochberg correction following a significant Kruskal–Wallis test. ****, $p < .0001$; ***, $p < .001$; **, $p < .01$; *, $p < .05$; ns, not significant.

The pro-inflammatory cytokine TNF is the key driver of inflammation in many chronic inflammation settings and is secreted by various cell types, including macrophages, T cells, B cells, and NK cells.[45] Inhibition of TNF by a variety of TNFi suppressed the development of RBD-specific IgG4 after third mRNA vaccination. Total IgG anti-RBD levels were comparable to those of healthy and untreated DC, indicating a specific block in IgG4 switching in these patients. Since B cells do not express receptor for TNFR1, the observed impact of blocking TNF on IgG4 skewing implies an indirect effect via other immune cells. Interestingly, several TNFi (adalimumab in particular) have been extensively studied in light of anti-drug antibody development, a typical IgG4-skewed response. In these responses, adalimumab treatment has been shown to induce potent neutralizing anti-drug antibodies that shift towards IgG4.[46] It is somewhat paradoxical that substantial IgG4 skewing of these anti-adalimumab antibodies is observed under TNF blockade, while IgG4 skewing of the mRNA vaccination-induced immune response is markedly reduced. Notably, total serum IgG4 levels are not affected by adalimumab treatment in general.[25] The magnitude of IgG4 switching may be dependent on a delicate balance between multiple pathways, whereby the nature of the antigen itself might be a key factor in directing this balance. The possibility for multiple signaling routes contributing to IgG4 switching, to various extents, might be reflected in the observation that a subset of individuals still demonstrated substantial IgG4 skewing despite blocking of either IL-4/IL-13 or TNF. However, a complete understanding of the mechanistic drivers responsible for IgG4 switching is still lacking.

The extent to which IgG4 class switching will affect immune protection to SARS-CoV-2 remains unclear, yet most likely depends on the different modes of action of antibodies during SARS-CoV-2 infection and vaccination. One mechanism of protection involves neutralization of the pathogen by interfering with the interaction of the spike protein to ACE2. In general, neutralizing antibody titers induced by SARS-CoV-2 infection or mRNA vaccination correlate well with protection from infection,[47–49] and IgG4 was found to have good neutralizing capacity.[15] Effector functions mediated by the Fc tail of IgG have also been suggested to contribute to protection via complement-dependent or FcγR-dependent viral clearance.[50,51] On the other hand, Fc-mediated

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effector functions might also contribute to excessive inflammation, leading to a more severe disease course. High levels of proinflammatory afucosylated antibodies were for instance found in patients admitted to the intensive care unit (ICU) following SARS-CoV-2 infection, suggesting a potential pathogenic role for the latter.[52,53] Whether or not the weak potential of IgG4 to induce Fc effector functions is advantageous in this context remains to be determined.[54] More broadly, even if the balance between direct neutralization (which IgG4 is highly capable of) opposed to effector function-mediated protection (which IgG4 is poor at) is such that for SARS-CoV-2 the IgG4 skewing does not significantly alter immune protection, this might work out differently for other viruses, and proper understanding of IgG4 skewing induced by mRNA vaccines as well as possible interventions to avoid this are relevant to explore further.

Overall we have demonstrated significantly reduced IgG4 class switching by dupilumab as well as TNFi upon repeated mRNA vaccination for SARS-CoV-2. In other words, this study provides *in vivo* evidence for both TNF as well as IL-4 and/or IL-13 being instrumental in IgG4 class switching.

AUTHOR CONTRIBUTIONS

AMV, FE, TWK, SMvH, ATB, and TR conceptualized and supervised the study. KPJvD, EWS, LW and LYLK curated clinical data with supervision from FE and TWK. JBDK, SK, NILD, MvD, GvM, MS and TR performed serological assays. JBDK curated serological data with supervision from MS and TR. AMV and JBDK visualized the data. AMV, JBDK and TR interpreted the data. AMV and JBDK wrote the first draft of the manuscript. All remaining authors participated in clinical data acquisition. All authors other than AMV and JBDK reviewed the manuscript.

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CONFLICT OF INTEREST STATEMENT

PIS is involved in clinical trials with many pharmaceutical industries that manufacture drugs used for the treatment of, for example, psoriasis and atopic dermatitis, for which financial compensation is paid to the department or hospital, and is a chief investigator of the TREAT NL registry taskforce and SECURE-AD registry. MWB is a secretary for the Dutch Experimental Dermatology Board; head of the pigmentary disorders group within the Dutch Dermatology Board; received grants/contracts from Novartis, Incyte, AbbVie, Vitiligo.nl; reports honoraria from Novartis, Abbvie, UCB, Sanofi for lectures on vitiligo and melanoma; and participation on an advisory board for Sanofi-Genzyme. BH reports unpaid positions as a medical adviser for several patient groups, a board position for ERN-SKIN, and associate editor for *The British Journal of Dermatology*; reports grants from AbbVie, Akari Therapeutics, Celgene, and Novartis; consulting fees from UCB Pharma, Novartis, and Janssen; and honoraria from AbbVie. DJH received payments to the institution through grants from UCB, LEO and for clinical trials for Abbvie, Almirall, Galderma, and Sanofi; consulting fees for Abbvie, Almiral, Galderma, Janssen, Lilly, Novartis, Pfizer, and Sanofi; declares unpaid board or councilor roles for ISAD, NVDV, IEC, ETFAD, and NVED. JK has speaking relationships with F. Hoffmann-La Roche Ltd, Biogen, Immunic, Teva, Merck, Novartis and Sanofi/Genzyme; received financial support to his institution for research activities from F. Hoffmann-La Roche Ltd, Biogen, Immunic, Teva, Merck, Novartis, Sanofi/Genzyme, and for a role in an adjudication committee of MS clinical trial of Immunic; and received grants for multicenter investigator initiated trials by ZonMW and Treatmeds. FE and TWK report (governmental) grants from ZonMw to study immune response after SARS-Cov-2 vaccination in autoimmune diseases. FE also reports grants from Prinses Beatrix Spierfonds, CSL Behring, Kedrion, Terumo BCT, Grifols, Takeda Pharmaceutical Company, and GBS-CIDP Foundation; consulting fees from UCB Pharma and CSL Behring; and honoraria from Grifols. All other authors declare no competing interests.

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public-private partnerships and co-financing by health foundations that are part of the SGF.

DATA AVAILABILITY STATEMENT

Anonymized individual data and a data dictionary will be made available under a data sharing agreement to researchers who provide a methodologically sound proposal to the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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