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BAFF-R Expression as a Potential Biomarker Associated with COVID-19 Vaccine Non-Responsiveness in Antibody-Deficient Patients

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Key words: BAFF-R; antibody deficiency; SARS-CoV-2 vaccination; vaccine non-responsiveness; B-cell differentiation

Abbreviations: BAFF-R, B-cell activating factor receptor; BAFF, B-cell activating factor; APRIL, a proliferation-inducing ligand; PC, plasma cell; ASC, antibody-secreting cell; PBMC, peripheral blood mononuclear cell; HC, healthy control; PAD, primary antibody deficiency; SAD, secondary antibody deficiency; SPAD, specific polysaccharide antibody deficiency; CVID, common variable immunodeficiency; COV-AD, COVID-19 in Antibody Deficiency; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; IGH, immunoglobulin heavy chain; BCR, B-cell receptor; TACI, transmembrane activator and CAML interactor; BCMA, B-cell maturation antigen; CLL, chronic lymphocytic leukaemia;

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CDR3, complementarity-determining region 3; ESID, European Society for Immunodeficiencies; IMDM, Iscove's Modified Dulbecco's Medium; FBS, fetal bovine serum; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; RNA-seq, RNA sequencing; TPM, transcripts per million; RFU, relative fluorescence units; TNFRSF13C, tumour necrosis factor receptor superfamily member 13C; TNFSF13B, tumour necrosis factor ligand superfamily member 13B.

Abstract

Introduction: Patients with primary and secondary antibody deficiencies exhibit variable responses to vaccination, with many failing to mount optimal immunity to SARS-CoV-2. Mechanisms underpinning vaccine non-responsiveness remain poorly defined and unpredictable. We hypothesised that B-cell-intrinsic features are associated with SARS-CoV-2 vaccine failure.

Methods: Peripheral B-cells from 49 patients enrolled in the COVID-19 in Antibody Deficiency (COV-AD) study underwent a validated *in vitro* B-cell differentiation assay. We assessed plasmablast and plasma cell (PC) generation, immunoglobulin production, immunoglobulin heavy chain (IGH) repertoire diversity, and BAFF-R expression.

Results: Vaccine non-responders displayed reduced IgA class-switched immunoglobulin production *in vitro* compared to healthy controls and responders. Moreover, while the relative percentage of PC output was comparable between groups, the overall number of cells obtained from non-responders was reduced. Most non-responders and a subset of responders exhibited reduced BAFF-R surface expression at baseline compared to healthy controls, though with considerable overlap between groups. BAFF-R transcript levels partially corresponded with surface expression but varied and did not clearly distinguish response. No compensatory upregulation of alternative BAFF receptors or elevated serum BAFF was observed. IGH repertoire analysis revealed preserved diversity among patients.

Conclusions: Diminished BAFF-R expression is associated with vaccine non-responsiveness and may indicate underlying B-cell-intrinsic defects. BAFF-R shows potential as a candidate biomarker that merits further validation in larger, multicentre cohorts to determine its clinical utility for stratifying patients at risk of vaccine failure. These findings suggest that the BAFF/BAFF-R axis may play an important role in vaccine-induced humoral immunity in antibody-deficient patients, warranting further mechanistic investigation.

Introduction

B-cells are crucial for humoral immunity, producing antigen-specific antibodies through tightly regulated differentiation pathways. Defects in B-cell differentiation, observed in primary (PAD) and secondary (SAD) antibody deficiencies, can impair immune responses and increase infection susceptibility^{1,2}. PADs, such as Common Variable Immunodeficiency (CVID), are driven by intrinsic immune defects, which are genetically defined in some cases but remain idiopathic in many instances³. In contrast, SADs result from external factors, typically malignancies or immunosuppressive therapies that impair B-cell function^{3, 4}.

The COVID-19 pandemic highlighted the vulnerability of these patients, with approximately one quarter of immunodeficient individuals who contracted SARS-CoV-2 dying during the initial wave⁵. While antibody deficiency is often assumed to preclude vaccine responsiveness, this is not universally the case^{6,7}. Differentiating responders from non-responders within well-characterised antibody deficient cohorts presents an opportunity to uncover immunological factors necessary for protection, challenging long-held assumptions regarding vaccine failure in this population.

The COVID-19 in Patients with Antibody Deficiency (COVAD) study is a multi-site initiative, encompassing heterogeneous cohorts of both SAD and PAD cases^{5,6,7}. Initial data revealed that nearly a quarter of patients failed to respond to three vaccine doses, with clinical factors such as bronchiectasis, splenomegaly, liver disease, and immunosuppressive therapy potentially contributing to non-response. Despite these insights, a significant gap remains in understanding factors driving vaccine response versus non-response.

The present study aims to characterise B-cell intrinsic responses using a validated *in vitro* B-cell differentiation model. By identifying defects in B-cell pathways, we seek to uncover factors that distinguish vaccine responders from non-responders, enhancing our understanding of vaccine failure in patients.

Herein, we report that non-responders generated comparable proportions of plasma cells (PC) to responders but had reduced IgA class-switched immunoglobulin *in vitro*. Additionally, we identify reduced BAFF-R expression as a feature associated with SARS-CoV-2 vaccine non-responsiveness in antibody-deficient patients. These discovery-phase findings support BAFF-R as a candidate biomarker warranting further validation in larger, multicentre cohorts to determine its clinical utility for stratifying patients at risk of vaccine failure.

METHODS

Sample collection

Peripheral blood mononuclear cell (PBMC) samples were obtained from healthy donors and patients enrolled in the COV-AD study with primary and secondary antibody deficiencies after informed consent^{5,6,7}. Samples were collected within 28 days following the third or fourth dose of the SARS-CoV-2 vaccine. Patients were eligible if they were ≥ 18 years old and either receiving immunoglobulin replacement therapy or had a serum IgG < 4 g/L with antibiotic prophylaxis. Diagnoses were assigned using European Society of Immunodeficiencies (ESID) Clinical Working Party criteria. CVID was defined as hypogammaglobulinaemia with reduced serum IgG and IgA and/or IgM, impaired antibody production following vaccination and/or infection, and exclusion of secondary causes. Hypogammaglobulinaemia was defined as persistently reduced IgG (with or without low IgA/IgM) not meeting full ESID criteria for CVID. Specific polysaccharide antibody deficiency (SPAD) was defined as impaired response to polysaccharide vaccines despite normal total immunoglobulin levels. Secondary antibody deficiency (SAD) was attributed to defined causes such as rheumatological disease or renal pathology (Table 1).

All patient samples were cryopreserved prior to analysis, as fresh PBMCs were not available. This approach was supported by in-house optimisation and validated using matched fresh and cryopreserved PBMCs from three independent healthy controls (HCs), which showed no discernible differences in terms of phenotype, isotype expression or cell number (Supplementary Figure 1). This is also consistent with published work⁸, reporting minimal effects of cryopreservation on B-cell function. Fresh HC PBMCs were used in the main assay to enable timely processing.

For patient samples, PBMCs were isolated within 24 h of collection using Leucosep tubes pre-filled with Lymphoprep (StemCell Technologies) or, where necessary, Ficoll-Paque PLUS (VWR International). PBMCs were resuspended in freezing medium (90% heat-inactivated FBS and 10% DMSO), aliquoted at $5\text{--}10 \times 10^6$ cells/ml, and frozen in a CoolCell controlled-rate freezing container at -80°C overnight before transfer to liquid nitrogen ($\leq -150^\circ\text{C}$). Thawing was performed at room temperature until completely thawed, following by washing in Iscove's Modified Dulbecco's Medium (IMDM, Thermo Fisher) with 10% FBS before B-cell differentiation assays. HC PBMCs were processed fresh using comparable Lymphoprep isolation methods.

Details on patient recruitment and sample collection are available in prior reports^{5,6,7}. Ethical approval was granted by London-Dulwich Research Ethics Committee (21/LO/0162) and Leeds East Research Ethics Committee (07/Q1206/47).

B-cell differentiation

B-cells were isolated from PBMCs using a Miltenyi B-cell isolation kit according to the manufacturer's protocol. This protocol first depletes non-B cells using a negative selection cocktail resulting in a total B-cell preparation. For HC samples in direct comparison experiments, the separation of total B-cells was followed by enrichment of naïve B-cells via anti-CD27 magnetic bead depletion (Miltenyi Biotec), yielding a predominantly CD19⁺CD27⁻ population as confirmed by flow cytometry.

B-cells were cultured at 2.5×10^5 cells/ml in Iscove's Modified Dulbecco's Medium (IMDM) containing 10% FBS. For activation at day 0, cells were stimulated with 20 U/ml human IL-2 (Miltenyi), 50 ng/ml human IL-21 (Peprotech), and 10 µg/ml F(ab')₂ goat anti-human IgM/IgG/IgA (Stratech), and co-cultured with irradiated murine fibroblasts expressing CD40L (CD40L-L cells). In some experiments, 100 ng/ml BAFF (AdipoGen), the upper range recommended for B-cell activation and differentiation⁹, was also included.

On day 3, cells were removed from CD40L-L cells and reseeded in fresh media supplemented with MEM amino acid solution (1:50) and lipid mixture 1 (1:200) (Sigma), containing 20 U/ml IL-2 and 50 ng/ml IL-21. Where indicated, culture media at day 3 also included 100 ng/ml BAFF (AdipoGen). At day 6, cells were reseeded in media supplemented with 10 ng/ml human IL-6 (Peprotech), 10 ng/ml IL-21, and 100 ng/ml APRIL (R&D Systems Biotechne). Cultures were terminated at day 13 to assess the generation of PCs.

Flow cytometry

Immunophenotyping of B-cells was performed using the following antibodies: CD19-PE (LT19, Thermo Fisher Scientific), CD20-eFluor 540 (2H7, Thermo Fisher Scientific), BAFF-R-BV421 (11C1, BD Biosciences), CD27-FITC (MT271, BD Biosciences), CD38-PE-Cy7 (HB7, BD Biosciences), and CD138-APC (MI15, Miltenyi). B-cells were identified

as $CD19^+CD20^+CD38^{lo}CD27^{+/-}CD138^-$; plasmablasts as $CD19^+CD20^{lo}CD38^{hi}CD27^+CD138^-$; and mature PCs as $CD19^+CD20^{lo/-}CD38^{hi}CD27^+CD138^+$. These definitions refer to the proportion of cells expressing the relevant markers. Live/dead discrimination was conducted using Zombie UV (BioLegend). For intracellular immunoglobulin staining, cells were fixed, permeabilised, and stained with IgM-APC (G20-127, BD Biosciences), IgG-FITC (G18-145, BD Biosciences), and IgA-VioBlue (IS11-8E10, Miltenyi).

Cells were stained in MACS buffer (PBS with 0.5% BSA) and analysed on a Beckman Coulter CytoFlex LX flow cytometer. Doublets were excluded based on forward scatter area versus height, and dead cells were excluded using Zombie UV staining. Cell counts were quantified with CountBright™ Absolute Counting Beads (Invitrogen). Data were analysed using FlowJo version 10 (BD Biosciences) and Prism 10 (GraphPad).

Immunoglobulin secretion

Supernatants were analysed for IgM, IgG, and IgA using enzyme-linked immunosorbent assays (ELISA). ELISAs were performed with Human IgM ELISA Quantitation Set (E80-100), Human IgG ELISA Quantitation Set (E80-104), or Human IgA ELISA Quantitation Set (E80-102) (Bethyl Laboratories Inc) according to the manufacturer's instructions.

RNA analysis

RNA was extracted from cell suspensions using TRIzol (Invitrogen) and sequencing was performed by Novogene (at 20M reads per sample). Raw sequencing data was quality assessed using FastQC (v0.12.1) and trimmed of adapters using Cutadapt (v5.0). High-quality reads were mapped to GRCh38.p13 reference genome and gene counts were quantified using Salmon (v1.10.3)^{10,11}. Differential gene expression analysis was performed using DESeq2 (v1.24.0) in RStudio, and data was visualised using ggplot2 (v3.5.2)^{12,13}.

Immunoglobulin heavy-chain (IGH) repertoire analysis

IGH sequences were amplified via a two-step PCR process using BIOMED-2 primers¹⁴. Indexed libraries were prepared with Nextera reagents and sequenced using Illumina platforms. Raw reads were analysed with MiXCR software and V/J gene combinations were visualised using CIRCOS plots (<https://mk.bcgsc.ca/tableviewer/visualize/>).

BAFF quantification

Serum TNFSF13B (BAFF) levels were measured using the SOMAscan® assay (SomaLogic, Inc.) and reported in relative fluorescence units (RFU).

Statistical analysis

Statistical analyses were performed using Prism 10 (GraphPad), applying one-way ANOVA or Kruskal–Wallis test with Dunn’s multiple comparisons post hoc test, or Wilcoxon rank-sum test for pairwise comparisons, as appropriate. Statistical significance was defined as $p < 0.05$ (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

RESULTS

Patient cohort characteristics

For the characterisation of B-cells in the context of response to SARS-CoV-2 vaccination amongst antibody deficient individuals, samples were used from 49 of the patients enrolled in the COV-AD study. Patients selected for *in vitro* differentiation analysis were chosen based on the availability of detailed metadata (Table 1), prioritising patients with confirmed presence of B-cells to assess potential B-cell intrinsic defects. The selected cohort comprised a heterogeneous group of both SARS-CoV-2 vaccine responding ($n=34$) and non-responding ($n=15$) patients with various primary ($n=29$) and secondary ($n=20$) antibody deficiencies. Samples were nationally obtained, and patients were both male and female adults, aged 24–78 (Table 1 and 2). Previous findings from the COV-AD study primarily define non-responders based on their inadequate serological response following a second dose of either the approved Pfizer BioNTech (162b2) or AstraZeneca (ChAdOx1 nCoV-19) vaccine. Specifically, non-responders had an IgGAM ratio < 1.0 , measured 1–2 months post-vaccination using anti-spike glycoprotein antibody assays¹⁵. Non-responders also lacked neutralising antibody activity and showed poor T-cell responses^{6,7}. Ten naïve-enriched B-cell samples from HCs were included in parallel. Naïve-enriched B-cells from HCs were used for most experiments, as the memory B-cell compartment was reduced or absent in many patients, although proportions varied across diagnoses (Supplementary Figure 2A). Furthermore, a comparison of total versus naïve-only B-cells at day 0 confirmed that variation in memory B-cell frequencies did not account for differences in baseline phenotype or differentiation outcomes (Supplementary Figure 2B–D).

BAFF increases *in vitro* cell numbers without impacting phenotype

Initial data from the COV-AD cohort revealed low B-cell numbers in the peripheral blood of patients, likely reflecting a combination of underlying immunodeficiency and sample cryopreservation. This was particularly marked in non-responders, who exhibited

pronounced peripheral B-cell lymphopenia on average, despite some individuals falling within the anticipated healthy range of $0.2\text{--}0.6 \times 10^9/\text{L}$ (Supplementary Fig 3A). This raised concerns about the feasibility of *in vitro* differentiation, as the minimal B-cell count required for productive differentiation using this system (Fig 1A) had not been established.

Titration experiments using fresh HC PBMCs demonstrated that CD19⁺ B-cells could survive and undergo differentiation into immunoglobulin-producing CD38^{hi}CD27⁺ plasmablasts and CD38^{hi}CD138⁺ PCs, even when seeded at reduced input numbers using a constant B-cell to CD40L-fibroblast ratio (Supplementary Fig 3B–E; Supplementary Table 1). While output was proportionally reduced (Supplementary Fig 3F), the phenotypic profile of differentiated cells was maintained, indicating that the assay remained functional despite lower starting cell numbers. However, this optimisation was performed on fresh HC samples and may not fully account for the additional impact of cryopreservation on patient-derived PBMCs.

BAFF, a TNF-family cytokine essential for B-cell survival, engages BAFF-R, TACI, and BCMA to modulate B-cell activation and differentiation¹⁶. Given its role in enhancing B-cell survival *in vitro*^{17,18,19}, we hypothesised that BAFF supplementation could increase patient B-cell recovery without altering phenotype, thereby improving assay reproducibility.

To test this, total B-cells from HCs were stimulated under three conditions: (1) BAFF added to pre-activated B-cells at day 0, (2) BAFF added at day 3 following activation, and (3) no BAFF, in keeping with the validated standard protocol^{20,21,22} (Figure 1B). CD38, CD138, CD27, CD19, and BAFF-R expression were assessed to confirm B-cell differentiation and evaluate BAFF-mediated effects.

At day 0, pre-activated B-cells exhibited a uniform CD19⁺BAFF-R⁺ phenotype. By day 6, BAFF-treated cultures, particularly those with day 3 BAFF addition, retained higher levels of BAFF-R expression, though surface expression was subsequently downregulated as differentiation progressed. Despite donor-specific variability, phenotypic markers and intracellular immunoglobulin expression followed the expected differentiation patterns across HCs (Figure 1C–F). Secreted immunoglobulin levels were largely comparable (Figure 1G), although day 3 BAFF addition was associated with modestly higher class-switched immunoglobulin levels at day 13. These findings were recapitulated in naïve-enriched B-cell culture (Supplementary Fig 4A–C).

Enumeration of cell numbers demonstrated the expected B-cell-expanding effect of BAFF, with BAFF-treated cultures exhibiting higher cell recovery compared to untreated cultures. Notably, the greatest expansion was observed in cultures supplemented with BAFF at day 3, a result that was consistently replicated across all three samples (Figure

1H). This finding was further validated in naïve-enriched B-cell cultures (Supplementary Fig 4D), reinforcing the reproducibility and efficacy of 100 ng/ml BAFF addition at this timepoint. Accordingly, BAFF supplementation at day 3 was applied to patient-derived samples to optimise cell recovery in subsequent experiments.

Patient samples generate plasmablasts and plasma cells *in vitro*

Investigating the inherent differentiation capacity of B-cells is essential to understanding whether non-responders fail to produce sufficient PCs, possibly contributing to poor vaccine responses. Flow cytometry phenotyping was conducted on all 49 samples using our *in vitro* differentiation model with data from two representative samples per group (HCs, responders, and non-responders) shown in detail (Table 1).

The representative HCs displayed the expected gradual increase in CD27⁺ cells, reaching >95% CD38^{hi}CD27⁺ by day 13, which encompasses both plasmablasts and PCs. This pattern was also observed in representative patient samples, albeit to varying extents (Figure 2A). While all representative samples showed an overall increase in CD38^{hi}CD27⁺ populations, the majority exhibited lower frequencies of these cells compared with controls at day 6 in both responders and non-responders. By day 13, a similar trend was observed; however, statistical significance persisted only between controls and non-responders. The magnitude of this difference was smaller than at day 6, and considerable inter-patient variability was evident in both patient groups (Figure 2B).

Across the cohort, no significant differences in the percentages of CD138⁺ PCs were observed between controls, vaccine responders, and non-responders (Figures 2C–D). Although representative CVID patients showed reduced PC generation compared to controls, this was not consistently linked to vaccine response status (Figure 2C).

Collectively, phenotypic data examining PC/plasmablast generation show that, despite differences indicating reduced differentiation capacities across all COV-AD samples, both vaccine response groups were able to generate similar proportions of CD38^{hi}CD27⁺ plasmablasts and CD38^{hi}CD27⁺CD138⁺ PCs.

Nevertheless, patient cultures showed reduced recovery overall, with non-responders exhibiting significantly lower cell counts compared to controls at both time points when antibody-secreting cells are generated (Figure 2E).

***In vitro*-generated patient ASCs produce reduced levels of class-switched immunoglobulins**

The loss or failure of immunoglobulin function, particularly IgG, is a hallmark of antibody deficiency¹. Given evidence linking impaired immunoglobulin class-switching to diminished SARS-CoV-2 vaccine responses^{23,24}, we investigated whether these findings extended to our cohort by assessing intracellular and secreted IgM, IgG, and IgA expression.

Representative controls exhibited the expected intracellular immunoglobulin profile, with high IgM at day 6 that declined by day 13, alongside increased IgG and IgA (Figure 3A). In contrast, patient samples exhibited persistently elevated IgM and reduced IgG and IgA expression at both time points. Both responders and non-responders had significantly higher IgM and lower IgA expression than controls; for IgG, both patient groups showed lower expression than controls, but only IgA was significantly reduced in non-responders compared to responders (Figure 3B), a trend not accounted for by baseline memory B-cell frequencies, which were comparable between groups (S2C and D). This suggests that reduced induction of IgA *in vitro*, rather than with clinical IgA deficiency itself, may be associated with poor SARS-CoV-2 vaccine response in antibody-deficient patients²⁵.

Trends in immunoglobulin secretion largely mirrored intracellular findings. Both patient groups had significantly lower IgG and IgA secretion than controls by day 13. While non-responders showed a tendency toward increased IgM, no statistically significant differences in secreted IgM, IgG, or IgA were observed between responders and non-responders (Figure 3C).

Patient samples generate polyclonal plasma cells *in vitro*

The human immune repertoire relies on diverse V(D)J segment combinations within the immunoglobulin heavy chain (IGH) locus to generate antibodies against a wide range of pathogens. Given this, vaccine non-responders may exhibit alterations in IGH V-J segment usage, potentially limiting repertoire diversity. To investigate this, IGH V-J gene segment combinations were compared between selected vaccine-responding and non-responding patients (P5–10, Table 1) using sequences obtained from day 13 *in vitro*-differentiated cultures of total B-cells isolated from patients.

Consistent with previous reports of preserved IGH repertoire diversity in antibody-deficient patients²⁶, all patient samples exhibited broadly distributed V-J pairings (Figure 4A), indicating retained immunoglobulin gene usage regardless of vaccine response. Whilst no major differences were observed between responders and non-responders, P18 showed highly restricted V-J pairings, likely reflecting an underlying diagnosis of chronic lymphocytic leukaemia (CLL) that was retrospectively identified during analysis (Supplementary Figure 5). This underscores the utility of the system in flagging patients

1 with markedly impaired B-cell function, including those with underlying haematological
2 malignancy.

3 To further explore whether IGH repertoire characteristics could distinguish vaccine
4 responders from non-responders, we examined the variable heavy chain
5 complementarity-determining region 3 (CDR3) length and net charge, as these features
6 can influence antigen recognition and may reflect repertoire immaturity or autoreactivity²⁷.
7 CDR3 lengths and charges appeared elevated in some non-responders compared to the
8 rest of the cohort; however, there was considerable variability amongst all patients, and
9 the small sample size was insufficient for meaningful statistical analysis (Supplementary
10 Fig. 6A and 6B).

11 These findings suggest that IGH diversity, both at the level of V-J recombination and basic
12 CDR3 physicochemical properties, is generally preserved in vaccine non-responders.
13 Functional defects in these patients are therefore more likely to result from downstream
14 mechanisms, such as signalling defects or impaired maturation, rather than from
15 constraints in repertoire generation²⁸.

16

Vaccine non-responders have significantly reduced or absent BAFF-R expression on B-cells

To further explore whether non-responders might have alterations in key external regulators of the B-cell lineage, we assessed the proportion of CD19⁺ B-cells expressing BAFF-R^{28,29,30}. In line with expected patterns³¹, naïve B-cells from HCs exhibited a high proportion of BAFF-R⁺ cells at day 0. This proportion declined by day 6 and remained low at day 13, consistent with differentiation-associated downregulation (Figure 5A).

In contrast, most vaccine non-responders displayed a lower proportion of BAFF-R⁺ total B-cells at day 0 compared to HCs and the majority of responders. By day 6, BAFF-R expression had decreased in all groups, consistent with differentiation-associated downregulation, and non-responders maintained a lower proportion of BAFF-R⁺ B-cells throughout culture. A subset of responders also exhibited low BAFF-R expression at day 0 (Figure 5A–B), an observation that did not correlate with borderline IgGAM values (Table 1) or with consistent differences in cell phenotype, frequency, or antibody secretion during *in vitro* differentiation.

Given the differences in BAFF-R⁺ proportions at day 0, RNA was collected from matched samples at this timepoint to determine whether transcriptional dysregulation of *TNFRSF13C*, the gene encoding BAFF-R, contributed to the phenotype. Selected samples represented both CVID and SAD patients, spanning vaccine responders and non-responders with a range of BAFF-R expression. Low BAFF-R expression was defined as $\leq 50\%$ of the HC median. RNA sequencing revealed reduced *TNFRSF13C* transcript abundance in both responders and non-responders with low BAFF-R surface expression, suggesting that diminished transcription may contribute to the phenotype (Figure 5D). However, *TNFRSF13C* expression was variable within each group, including among HCs, indicating that post-transcriptional or other regulatory mechanisms may also be involved.

Reduced BAFF-R expression has been described in antibody deficiencies, particularly CVID, where it can inversely correlate with serum BAFF levels and associate with increased TACI expression³². We therefore hypothesised that reduced BAFF-R expression in non-responders might be linked to elevated serum BAFF, leading to increased expression of alternative receptors TACI and BCMA. To explore this, BAFF-R, TACI, and BCMA expression was assessed at days 0, 6, and 13. Although reductions in BAFF-R expression were confirmed, TACI and BCMA followed expected differentiation-associated patterns in all groups³², with no evidence of compensatory upregulation in patients with low BAFF-R expression at day 0 (Supplementary Figure 7).

Finally, to test whether impaired BAFF-R expression was associated with elevated serum BAFF, serum BAFF concentrations (TNFSF13B) were measured. No significant differences were observed between groups (Figure 5E), suggesting that systemically elevated BAFF is unlikely to account for reduced BAFF-R expression in this cohort.

DISCUSSION

The vulnerability of antibody-deficient patients to severe infections, notably COVID-19, highlights the critical need to better understand the immune dysfunctions underlying these conditions. Despite vaccination efforts, many antibody-deficient individuals remain at heightened risk of poor outcomes due to impaired capacity to generate adequate immune responses. This issue remains especially pertinent in the context of COVID-19 and the emergence of new variants, as well as other infectious diseases that continue to threaten immunocompromised populations.

This study provides novel insights into B-cell-intrinsic features associated with vaccine non-responsiveness, with a particular focus on BAFF-R expression. Using an optimised *in vitro* B-cell differentiation model^{20,21,22}, we found that non-responders exhibited significantly reduced total cell numbers compared to HCs and reduced IgA class-switched immunoglobulin production *in vitro* compared to HCs and responders. The most consistent immunophenotypic difference was a lower proportion of BAFF-R⁺ B-cells at baseline in non-responders, with similar patterns observed in a subset of responders.

Impaired BAFF-R expression has previously been associated with heightened disease activity in immunocompromised individuals, including those with COVID^{32,33,34,35}. Reduced BAFF-R availability has been linked to elevated circulating BAFF levels and compensatory upregulation of alternative receptors such as TACI and BCMA^{32,36,37,38}. However, this was not observed in our cohort. Patients with diminished BAFF-R⁺ proportions did not show accompanying increases in TACI or BCMA, but did exhibit relatively lower transcript levels of *TNFRSF13C*. In addition to mRNA abundance, surface receptor availability may be influenced by post-transcriptional regulation, mRNA stability, receptor shedding, or altered protein trafficking^{39,40,41}.

These observations support a model in which diminished BAFF-R expression reflects a multifactorial interplay of genetic, epigenetic, and environmental factors^{41,42,43}, rather than a single pathway defect. While our findings demonstrate an association between reduced BAFF-R⁺ proportions and vaccine non-responsiveness, causality cannot be inferred from this study. Nevertheless, BAFF-R remains a promising candidate biomarker that warrants further evaluation for its potential to stratify patients at higher risk of poor vaccine responses, potentially informing the use of additional vaccine doses or passive immunisation approaches.

Our results align with recent evidence implicating the BAFF/BAFF-R axis in vaccine efficacy. For example, Faliti et al. (2024)⁴⁴ reported that anti-BAFF therapy correlated with reduced vaccine-induced antibody responses, supporting the view that BAFF signalling is important for B-cell survival and differentiation. The association between reduced BAFF-R expression and altered differentiation trajectories in our model suggests that impaired signalling, whether from reduced receptor expression or downstream defects, may contribute to suboptimal humoral immunity in antibody-deficient individuals. However, the exact mechanistic links between BAFF-R dysfunction and impaired vaccine responses remain incompletely defined.

1 Future work should focus on delineating the molecular mechanisms regulating BAFF-R
2 expression and function, and on understanding how these influence short- and long-term
3 vaccine efficacy. Larger, multicentre studies that include a wide range of primary and
4 secondary immunodeficiencies will be essential to determine whether specific conditions
5 are disproportionately affected and to validate these findings. Such studies should use
6 fresh samples with a more homogeneous starting B cell population to minimise technical
7 variability and strengthen mechanistic insights. Extending these investigations to
8 vaccines against other pathogens will help establish whether the observed associations
9 reflect broader principles of impaired vaccine responsiveness.

10 By integrating mechanistic analysis with the evaluation of clinically
11 relevant candidate biomarkers, this work lays the groundwork for developing more
12 personalised vaccine strategies that could improve immune outcomes and guide targeted
13 interventions for vulnerable patient groups.

14

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Table and Figure legends

Table 1: Clinical and immunological features of antibody-deficient patients of a cohort of patients enrolled in the COV-AD study (n=49). Summary of demographic data, diagnosis, immunoglobulin therapy, COVID-19 vaccine type, and serological responses post-dose 2 (V2) and 3 (V3), measured by anti-spike IgGAM ratio in patients with recoverable B-cells. Pre-treatment IgG, trough immunoglobulin levels (IgG, IgA, IgM), and lymphocyte subset counts (CD3, CD4, CD8, CD19, CD56) are also included. Diagnoses include common variable immunodeficiency (CVID), and secondary immunodeficiencies related to haematological (HAEM) or rheumatological (RHEUM) conditions, as well as other rare immunodeficiencies. Data shown for patients P1–P49 represent a subset of the COV-AD cohort included in the analysis. Black denotes vaccine responders and red denotes vaccine non-responders.

Table 2: Characteristics of patients enrolled in the COV-AD study in which total B-cells were successfully isolated. Response to SARS-CoV-2 vaccination as determined post third or fourth vaccination.

Figure 1: The addition of BAFF to activated total B-cells enhances cell counts without altering phenotype. (A) Schematic of standard *in vitro* B-cell differentiation assay. (B) Experimental setup for the evaluation of the temporal effects of B-cell activation factor (BAFF) on B-cell activation and differentiation. Total B-cells were isolated from a healthy control and the impact of 100 ng/ml BAFF addition at day 0 versus day 3 of culture, relative to no addition, was assessed. (C) Representative flow cytometry plots showing CD38^{hi}CD27⁺, CD38⁺CD138⁺ and CD19⁺BAFF-R⁺ expression following T-dependent stimulation. (D) Relative percentages and averages of CD38^{hi}CD27⁺, and CD38⁺CD138⁺ and CD19⁺BAFF-R⁺ populations gated from total B cells in three independent healthy controls (HCs, n=3) at days 0, 6, and 13 (D0, D6 and D13). Each plot represents the percentage of cells expressing the indicated marker; colours indicate different culture conditions. (E) Intracellular IgM, IgG, and IgA expression at D6 and D13 in a representative HC. (F) Relative percentages and averages of IgM⁺, IgG⁺, and IgA⁺ populations at D6 and D13 in three independent HCs (n=3). (G) Quantification of secreted IgM, IgG, and IgA under different BAFF conditions at D6 and D13. (H) Viable cell counts

in three independent HCs (n=3) during differentiation under no BAFF, day 0 BAFF, or day 3 BAFF conditions, shown as mean $\pm$ SD.

Figure 2. Vaccine-responding and non-responding antibody deficient patient total B-cells are capable of generating plasma cells *in vitro*. (A) Representative example of naïve B-cells isolated from healthy controls (HCs); CVID vaccine-responder; SAD vaccine-responder; CVID vaccine non-responder and SAD vaccine non-responder. B-cells were assessed at days 0, 6 and 13 for plasmablast and plasma cell generation by the gain of CD27 and CD38 after T-dependent stimulation conditions. (B) Quantification of the percentage of cells expressing CD38^{hi}CD27⁺ at day 6 and day 13. (C) Representative examples of naïve B-cells isolated from HCs; Common Variable Immunodeficiency (CVID) vaccine-responder; Secondary antibody deficient (SAD) vaccine-responder; CVID vaccine non-responder and SAD vaccine non-responder. B-cells were assessed at days 0, 6 and 13 for plasma cell differentiation by the gain of CD38 and CD138 after stimulation with T-dependent stimulation conditions. (D) Quantification of the percentage of cells expressing CD138 at day 6 and day 13. HC naïve B-cells, n=10; Vaccine-responding patient B-cells, n=34; Vaccine non-responding patient B-cells, n=15. Statistical significance was determined using one-way ANOVA or Kruskal-Wallis test with Dunn's multiple comparisons (* $p<0.0001$, *** $p<0.001$, * $p<0.01$, * $p<0.05$ **). (E) Total cell numbers per ml at day 6 and day 13 in HCs (n=10), vaccine-responding patients (n=34), and vaccine non-responding patients (n=15). Statistical significance was determined using one-way ANOVA or Kruskal-Wallis test with Dunn's multiple comparisons (* $p<0.0001$, *** $p<0.001$, * $p<0.01$, * $p<0.05$ **).

Figure 3: Vaccine-responding and non-responding antibody deficient patients show no discernable difference in immunoglobulin expression. (A) Representative examples of intracellular IgM, IgG and IgA expression in naïve B-cells isolated from healthy controls (HCs); CVID vaccine-responding; SAD vaccine-responding; SAD vaccine non-responding and CVID vaccine non-responding patients at D6 and D13. (B) Assessment of the percentage of cells expressing IgM, IgG and IgA at D6 and D13. HC naïve B-cells, n=10; vaccine-responding patient B-cells, n=33; vaccine non-responding patient B-cells, n=14. (C) Secreted IgM, IgG and IgA expression in HCs, vaccine-responding and vaccine non-responding patients. Statistical significance was determined using one-way ANOVA or Kruskal-Wallis test with Dunn's multiple comparisons (* $p<0.0001$, *** $p<0.001$, * $p<0.01$, * $p<0.05$ **).

Figure 4: Vaccine-responding and non-responding antibody deficient patients show no discernable difference in V-J recombination. V and J gene usage in COV-AD patients (P5-P10) from BCR sequences obtained from RNA extracted at D13 of *in vitro* differentiation. Samples include SARS-CoV-2 vaccine-responders (n=3) and non-responders (n=3). Arc lengths represent the relative frequency of V or J gene usage, and

1 ribbon widths depict clonotype connection frequencies. Colours indicate distinct
2 clonotypes, illustrating diversity and clonality within each sample.

3 **Figure 5: Vaccine non-responders have significantly reduced or absent BAFF-R**
4 **expression in B-cells. (A)** Representative plots showing BAFF-R and CD19 expression
5 at days 0, 6, and 13 under T-dependent stimulation in naïve B-cells isolated from healthy
6 controls (HCs), CVID vaccine-responders, SAD vaccine-responders, CVID vaccine non-
7 responders, and SAD vaccine non-responders. **(B)** Histograms illustrating BAFF-R
8 expression across timepoints in representative individuals. **(C)** Quantification of
9 CD19⁺BAFF-R⁺ B cells at day 0, day 6, and day 13. Naïve B-cells isolated from HCs,
10 n=10; vaccine-responders, n=34; vaccine non-responders, n=15. **(D)** Gene expression of
11 *TNFRSF13C* (encoding BAFF-R) in naïve B-cells isolated from HCs (n=2), vaccine-
12 responders with high (n=5) and low (n=3) surface BAFF-R expression at day 0, and
13 vaccine non-responders (n=2) shown in transcripts per million (TPM). **(E)** Serum
14 TNFSF13B/BAFF levels in HCs, vaccine-responders, and vaccine non-responders
15 measured in relative fluorescence units (RFU). Statistical significance was determined
16 using one-way ANOVA or Kruskal-Wallis test with Dunn's multiple comparisons
17 (* $p < 0.0001$, *** $p < 0.001$, * $p < 0.01$, * $p < 0.05$ **).

19 Supplementary Tables and Figures

20 **Supplementary Figure 1: Fresh and cryopreserved PBMCs show no discernible**
21 **differences in B-cell phenotype, differentiation or expansion outcomes.**
22 **(A)** Comparison of BAFF-R, CD27, CD38, and CD138 expression on naïve-isolated B-
23 cells from matched fresh and cryopreserved PBMCs at day 0, day 6, and day 13 of
24 culture. Representative flow cytometry plots are shown alongside quantification of the
25 percentage of BAFF-R⁺, CD27⁺, CD38⁺, and CD138⁺ cells for each donor (n = 3).
26 **(B)** Analysis of IgM⁺, IgG⁺, and IgA⁺ B-cell differentiation at day 6 and day 13 for the same
27 fresh and cryopreserved samples. Representative plots and corresponding percentages
28 of IgM⁺, IgG⁺, and IgA⁺ cells are shown (n = 3). **(C)** Total viable cell number measured
29 across donors at day 0, day 6 and day 13 showing no notable difference in cell numbers
30 between fresh and cryopreserved cultures (n=3).

31 **Supplementary Figure 2: Baseline CD27⁺ frequency does not predict plasma cell**
32 **features during *in vitro* B-cell differentiation. (A)** Assessment of the percentage of
33 cells expressing CD27⁺ at day 0 (D0) following purification from PBMCs using non-B cell
34 depletion for patient samples or additional anti-CD27 beads for HCs. Data are displayed
35 on a logarithmic scale. HC naïve B-cells, n=10; vaccine-responding patient B-cells, n=34;
36 vaccine non-responding patient B-cells, n=15. **(B)** Representative flow cytometry plots
37 comparing CD27 and CD38 or BAFF-R expression in naïve-fractioned versus total B-cells

from a HC. **(C)** Four donors containing the highest percentage and four containing the lowest percentage CD27⁺ cells from either the HC or responder groups in part A are highlighted (purple = high CD27⁺, orange = low CD27⁺) and subsequently compared in panel D. The non-responder group did not contain sufficient samples with equivalent percentages so were not included. **(D)** Differentiation outcomes for CD27-high vs CD27-low donors analysed separately for HCs (left panels) and responders (right panels). Frequencies of CD38⁺CD27⁺ plasmablasts and IgA⁺ class-switched cells were quantified at day 6 and day 13, along with total viable cell number per ml. Statistical comparisons were performed using one-way ANOVA or Kruskal-Wallis with Dunn's multiple comparisons (**p*<0.05, ***p*<0.01, ****p*<0.001, *****p*<0.0001; *ns* = not significant).

Supplementary Figure 3: B-cell recovery and assay performance at limiting input cell numbers **(A)** CD19⁺ B-cell counts in peripheral blood mononuclear cells (PBMCs) from the COV-AD cohort, stratified by vaccine response status. Anticipated B-cell counts in healthy samples are typically in the range of 0.2–0.6 × 10⁹/L. **(B)** Whole blood from a representative healthy control (HC) was split into 15 ml, 10 ml, and 5 ml samples. Total B-cells were isolated and cultured under T cell-dependent (TD) conditions. Samples were analysed at days 0, 6, and 13 to assess the impact of reduced input on B-cell differentiation. **(C)** Relative percentages and averages of CD38^{hi}CD27⁺, CD138⁺ and CD19⁺CD20⁺ populations gated from total B-cells in three independent healthy controls (HCs, *n*=3) at days 0, 6, and 13 (D0, D6 and D13). Each plot represents the percentage of cells expressing the indicated marker; colours indicate different culture conditions **(D)** Relative percentages and averages of IgM⁺, IgG⁺, and IgA⁺ populations at D6 and D13 in three independent HCs (*n*=3). **(E)** Quantification of secreted IgM, IgG, and IgA under different conditions at D6 and D13. **(F)** Viable cell counts in three independent HCs (*n*=3) during differentiation under the different conditions, shown as mean ± SD.

Supplementary Table 1: Cell counts. Starting PBMC **(A)** and B-cell **(B)** numbers per ml in three different healthy controls (HC1, HC2 and HC3) from either 15 ml, 10 ml or 5 ml fresh blood.

Supplementary Figure 4: The addition of BAFF to activated naïve-fractionated B-cells enhances cell counts without altering phenotype. **(A)** Representative flow cytometry plots showing CD38^{hi}CD27⁺, CD38⁺CD138⁺ and CD19⁺BAFF-R⁺ expression following T-dependent stimulation at days 0, 6 and 13 (D0, D6 and D13) in naïve B-cells isolated from healthy control (HC) B-cells. **(B)** Intracellular IgM, IgG, and IgA expression at D6 and D13 in a representative HC. **(C)** Quantification of secreted IgM, IgG, and IgA under different BAFF conditions at D6 and D13. **(D)** Viable cell counts during differentiation in the representative control under no BAFF, day 0 BAFF, or day 3 BAFF conditions. Statistical significance was determined using one-way ANOVA or Kruskal-Wallis test with Dunn's multiple comparisons (**p*<0.0001, ****p*<0.001, **p*<0.01, **p*<0.05**).

Supplementary Figure 5: BCR repertoire analysis of a COV-AD SARS-CoV-2 vaccine-responding patient (P18) with CVID and concurrent CLL. Circos plot illustrating the dominant unique clonotype detected at D13 of *in vitro* differentiation. Arc lengths represent the relative usage of V and J gene segments; ribbon widths indicate clonotype connections.

Supplementary Figure 6: CDR3 length and charge in vaccine-responding and non-responding patients. (A) Mean CDR3 length (amino acids) from immunoglobulin heavy chain (IGH) repertoire analysis at day 13 in selected vaccine-responding (black) and non-responding (red) patients. (B) Mean CDR3 net charge for the same individuals. Each point represents the average value for all unique sequences within an individual. Groups were compared using a Wilcoxon rank-sum test, with no significant differences observed (length: $p = -1$; charge: $p = -1$).

Supplementary Figure 7: Reduced or absent BAFF-R expression is not accompanied by compensatory alterations in TACI or BCMA expression. Flow cytometric analysis of BAFF-R (B-cell activating factor receptor), TACI (transmembrane activator and CAML interactor), and BCMA (B-cell maturation antigen) on isolated B-cells from healthy controls (HCs, green), vaccine responders (grey/black), and vaccine non-responders (red) at day 0, day 6, and day 13 of culture.

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Not applicable.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request. Processed and raw RNAseq data will be publicly available at time of publication; Gene Expression Omnibus accession number: GSE298709.

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Conflicts of interest

Authors declare no conflict of interest

Ethical approval

1 Ethical approval was granted by London-Dulwich Research Ethics Committee
2 (21/LO/0162) and Leeds East Research Ethics Committee (07/Q1206/47). All study
3 participant provided signed informed consent statement

4 **Permission to reproduce**

5 Not applicable.

6 **Author contributions**

7 SS and GD conceived the study. EO conducted experiments. EO, LC, GD and SS
8 analysed the data. AMS, SOB and AGR provided patient samples, scientific direction,
9 contributed data and validated clinical information. EO and GD wrote the first draft of the
10 manuscript. All authors read, edited and approved the manuscript.

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Study identifier	Age	Sex	Diagnosis	Immunoglobulin (Product)	Vaccine administered	Post V2 IgAM ratio	Post V2 response	Post V3 IgAM ratio	Post V3 response	Pre treatment IgG (g/L)	Trough IgG (g/L)	IgA (g/L)	IgM (g/L)	Lymph (x10 ⁹ /L)	CD3 (x10 ⁹ /L)	CD4 (x10 ⁹ /L)	CD8 (x10 ⁹ /L)	CD19 (x10 ⁹ /L)	CD56 (x10 ⁹ /L)
P1	63	M	CVID	IVIG	AZ	3.71	Yes	6.14	Yes	3.3	9.2	0.9	0.8	0.576	0.51	0.424	0.077	0.039	0.016
P2	56	F	Secondary - HAEM	IVIG	Pfizer	5.53	Yes	20.39	Yes	2.6	7.8	0.01	0.5	0.838	0.761	0.489	0.254	0.01	0.07
P3	34	M	CVID	IVIG	AZ	0.13	No	n/a	n/a	n/a	9.8	n/a	n/a	0.925	0.699	0.362	0.322	0.075	0.123
P4	53	M	Secondary - HAEM	IVIG	AZ	0.31	No	4.16	Yes	0.5	9.1	0.1	0.1	0.953	0.826	0.52	0.271	0.01	0.099
P5	62	M	CVID	IVIG	AZ	1.90	Yes	6.17	Yes	6.1	8.5	0.1	0.5	1.028	0.781	0.289	0.426	0.229	0.01
P6	56	F	Secondary - RHEUM	SCIG	AZ	4.47	Yes	4.56	Yes	4.3	7.4	0.1	0.2	1.704	1.394	0.618	0.756	0.11	0.153
P7	34	F	CVID	IVIG	Pfizer	3.18	Yes	4.12	Yes	n/a	7.2	0.1	1.4	1.392	0.881	0.427	0.375	0.389	n/a
P8	34	F	Secondary - HAEM	None	AZ	0.18	No	0.14	No	0.1	n/a	0.01	0.01	2.1	1.89	0.874	0.809	0.12	0.115
P9	38	F	Secondary - HAEM	None	AZ	0.20	No	0.35	No	1.6	n/a	0	0	1.1	0.692	0.285	0.378	0.144	0.119
P10	69	M	CVID	IVIG	AZ	0.18	No	0.72	No	0.01	9	0.1	0.1	1.313	1.162	0.418	0.378	0.066	0.039
P11	62	M	CVID	SCIG	AZ	3.37	Yes	6.22	Yes	3.5	10	0	0.2	2.4	1.526	0.944	0.41	0.172	0.171
P12	64	F	Secondary - HAEM	IVIG	Pfizer	4.57	Yes	4.98	Yes	5.9	9.9	0.2	0.9	1.125	0.913	0.625	0.271	0.139	0.067
P13	72	F	Hypogammaglobulinaemia	IVIG	AZ	1.61	Yes	6.52	Yes	4.6	6.2	n/a	n/a	0.889	0.678	0.519	0.127	0.079	0.144
P14	78	F	CVID	IVIG	AZ	2.50	Yes	7.45	Yes	3.7	8.6	0.55	0.4	n/a	0.913	0.673	0.214	0.04	0.117
P15	76	F	Secondary - HAEM	IVIG	Pfizer	5.02	Yes	5.90	Yes	7	10.8	1.3	1.7	1.039	0.912	0.669	0.206	0.035	0.074
P16	60	M	CVID	IVIG	Pfizer	0.19	No	0.97	No	5.1	9.1	0.1	1	0.632	0.562	0.336	0.2	0.03	0.023
P17	59	F	Secondary - RHEUM	IVIG	Pfizer	1.30	Yes	1.61	Yes	5.7	10.5	0.5	0.1	0.681	0.335	0.127	0.193	0.003	0.311
P18	67	M	CVID	IVIG	AZ	3.36	Yes	7.12	Yes	n/a	6.6	0.2	0.2	2.241	0.76	0.579	0.155	1.765	1.765
P19	51	M	Secondary - HAEM	IVIG	AZ	1.06	Yes	3.16	Yes	1	3.42	0	0.03	2.14	1.2	0.61	0.43	0.81	0.12
P20	71	F	Secondary - HAEM	SCIG	AZ	4.65	Yes	8.76	Yes	5.09	13.1	1.3	0.7	1.51	0.62	0.37	0.24	0.51	0.37
P21	76	F	Secondary - RHEUM	IVIG	AZ	0.84	No	5.97	Yes	4.46	8.35	3.18	0.52	3.98	2.96	2.24	0.71	0.33	0.68
P22	35	M	Secondary - HAEM	SCIG	Pfizer	0.82	No	4.99	Yes	1.2	9.38	0	1	3.45	2.76	1.34	1.35	0.5	0.18
P23	31	F	CVID	None	AZ	0.29	No	0.74	No	3.55	n/a	0.22	0.26	2.48	2.35	1.34	0.92	0	0.11
P24	66	F	Secondary - HAEM	None	Pfizer	4.27	Yes	5.42	Yes	3.5	n/a	0.48	0.21	1.3	n/a	n/a	n/a	n/a	n/a
P25	24	F	STAT3 Hyper IgE	IVIG	AZ	1.56	Yes	3.67	Yes	n/a	9.9	0.7	0.5	1.739	1.273	0.819	0.435	0.348	0.058
P26	43	F	CVID	IVIG	AZ	1.32	Yes	3.51	Yes	1.3	7.9	0.1	0.3	1.957	1.511	0.67	0.781	0.256	0.199
P27	72	F	Secondary - HAEM	IVIG	AZ	1.92	Yes	5.97	Yes	n/a	6.6	0.2	2	0.253	0.204	0.052	0.153	0.016	0.03
P28	73	F	CVID	IVIG	AZ	16.23	Yes	21.41	Yes	n/a	12.9	0.9	0.4	1.404	1.042	0.707	0.315	0.203	0.119
P29	37	M	CVID	IVIG	AZ	2.81	Yes	5.30	Yes	3.9	7.7	0.2	0.3	0.645	0.485	0.308	0.157	0.102	0.048
P30	26	M	CVID	IVIG	AZ	1.07	Yes	0.83	No	4.2	10.1	0.9	0.6	0.742	0.574	0.384	0.181	0.039	0.083
P31	76	F	Hypogammaglobulinaemia	IVIG	Pfizer	5.25	Yes	6.62	Yes	6.4	10.8	0.9	4	1.496	1.103	0.903	0.181	0.064	0.247
P32	53	F	CVID	IVIG	Pfizer	1.53	Yes	0.37	No	n/a	8.7	0.1	0.1	1.699	1.174	0.609	0.471	0.564	0.035
P33	59	F	Secondary - HAEM	IVIG	Pfizer	0.21	No	0.80	No	n/a	9.8	0.1	0.1	10	1.648	1.367	0.307	10	0.036
P34	53	M	Secondary - RENAL	IVIG	AZ	4.88	Yes	5.17	Yes	0.7	4.9	0.4	0.1	1.524	1.056	0.612	0.452	0.092	0.288
P35	71	F	Hypogammaglobulinaemia	IVIG	AZ	2.31	Yes	4.35	Yes	5.1	9	0.7	0.2	0.641	0.532	0.43	0.1	0.054	0.04

P36	75	M	CVID	IVIG	Pfizer	3.86	Yes	1.34	Yes	n/a	12.9	0.1	0.1	1.8	1.119	0.414	0.675	0.199	0.486
P37	63	F	SPAD	IVIG	Pfizer	5.63	Yes	20.39	Yes	n/a	12.5	0.7	0.3	1.52	0.983	0.574	0.396	0.195	0.273
P38	43	M	CVID	IVIG	AZ	0.31	No	2.18	Yes	n/a	10	0.1	2.7	0.697	0.519	0.253	0.243	0.04	0.1
P39	38	F	Secondary - RHEUM	IVIG	Pfizer	1.65	Yes	0.84	No	5.9	10.9	0.1	0.1	1.328	0.989	0.41	0.521	0.02	0.291
P40	69	F	Secondary - RHEUM	IVIG	Pfizer	1.09	Yes	3.37	Yes	n/a	8.9	1.7	0.5	1.079	0.904	0.475	0.451	0.076	0.101
P41	50	F	CVID	IVIG	Pfizer	2.61	Yes	3.85	Yes	n/a	9.8	0.01	0.1	1.203	0.956	0.685	0.254	0.088	0.135
P42	61	F	Secondary - HAEM	IVIG	AZ	1.59	Yes	2.17	Yes	6.1	9.3	0.4	0.01	0.936	0.63	0.247	0.349	0.129	0.139
P43	74	F	CVID	IVIG	AZ	1.20	Yes	6.35	Yes	5	7.5	1	0.2	1.423	0.859	0.727	0.121	0.279	0.271
P44	59	F	Hypogammaglobulinaemia	IVIG	AZ	2.56	Yes	5.49	Yes	5	10.8	0.5	0.2	1.718	1.244	0.745	0.381	0.297	0.142
P45	33	F	CVID	SCIG	AZ	0.19	No	0.27	No	n/a	8.5	n/a	n/a	0.795	0.639	0.362	0.228	0.043	0.066
P46	70	M	CVID	IVIG	Pfizer	0.59	No	0.55	No	1.19	8.45	0.26	0.17	2.318	1.836	0.358	1.489	0.206	0.273
P47	56	F	CVID	IVIG	AZ	2.63	Yes	5.47	Yes	4.4	8.1	0.49	0.4	n/a	1.182	0.713	0.438	0.141	0.064
P48	56	F	CVID	SCIG	AZ	0.18	No	0.26	No	n/a	13.4	0	0	n/a	0.271	0.241	0.027	0.057	0.021
P49	74	M	Secondary - HAEM	IVIG	AZ	0.67	No	5.39	Yes	1.3	8.4	0.1	1.3	0.452	0.196	0.076	0.113	0.091	0.124

Table 1: Clinical and immunological features of antibody-deficient patients of a cohort of patients enrolled in the COV-AD study (n=49). Summary of demographic data, diagnosis, immunoglobulin therapy, COVID-19 vaccine type, and serological responses post-dose 2 (V2) and 3 (V3), measured by anti-spike IgGAM ratio in patients with recoverable B-cells. Pre-treatment IgG, trough immunoglobulin levels (IgG, IgA, IgM), and lymphocyte subset counts (CD3, CD4, CD8, CD19, CD56) are also included. Diagnoses include common variable immunodeficiency (CVID), and secondary immunodeficiencies related to haematological (HAEM) or rheumatological (RHEUM) conditions, as well as other rare immunodeficiencies. Data shown for patients P1–P49 represent a subset of the COV-AD cohort included in the analysis. Red denotes vaccine non-responders. Red denotes vaccine non-responders.

1

2 Table 2

	Overall	Vaccine responders	Vaccine non-responders
n	49	34	15
Primary antibody deficiency, n	29	21	8
Secondary antibody deficiency, n	20	13	7
Female, n	32	25	7
Male, n	17	9	8
Age range (years)	24–78	24–78	31–74

3

4

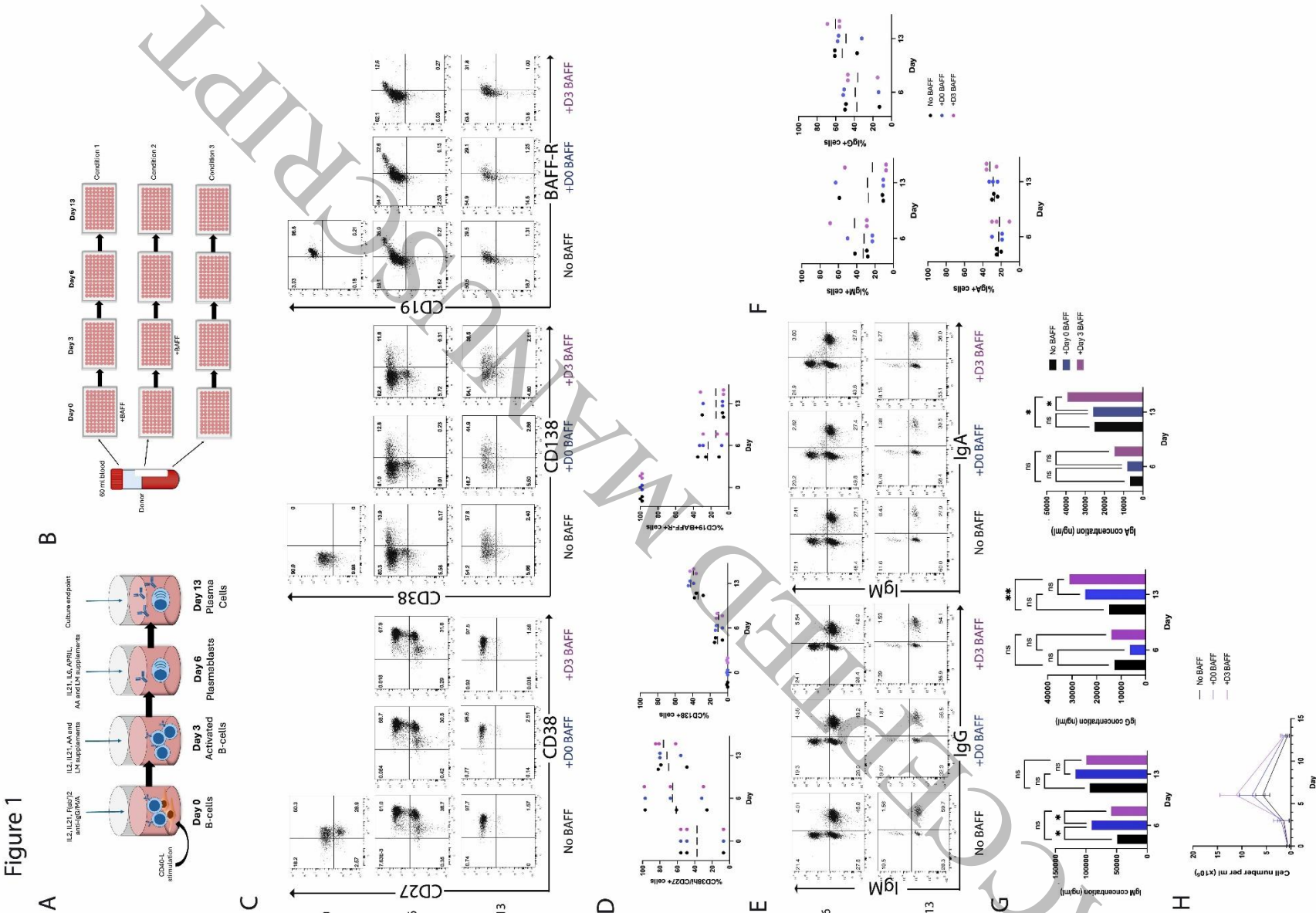


Figure 2

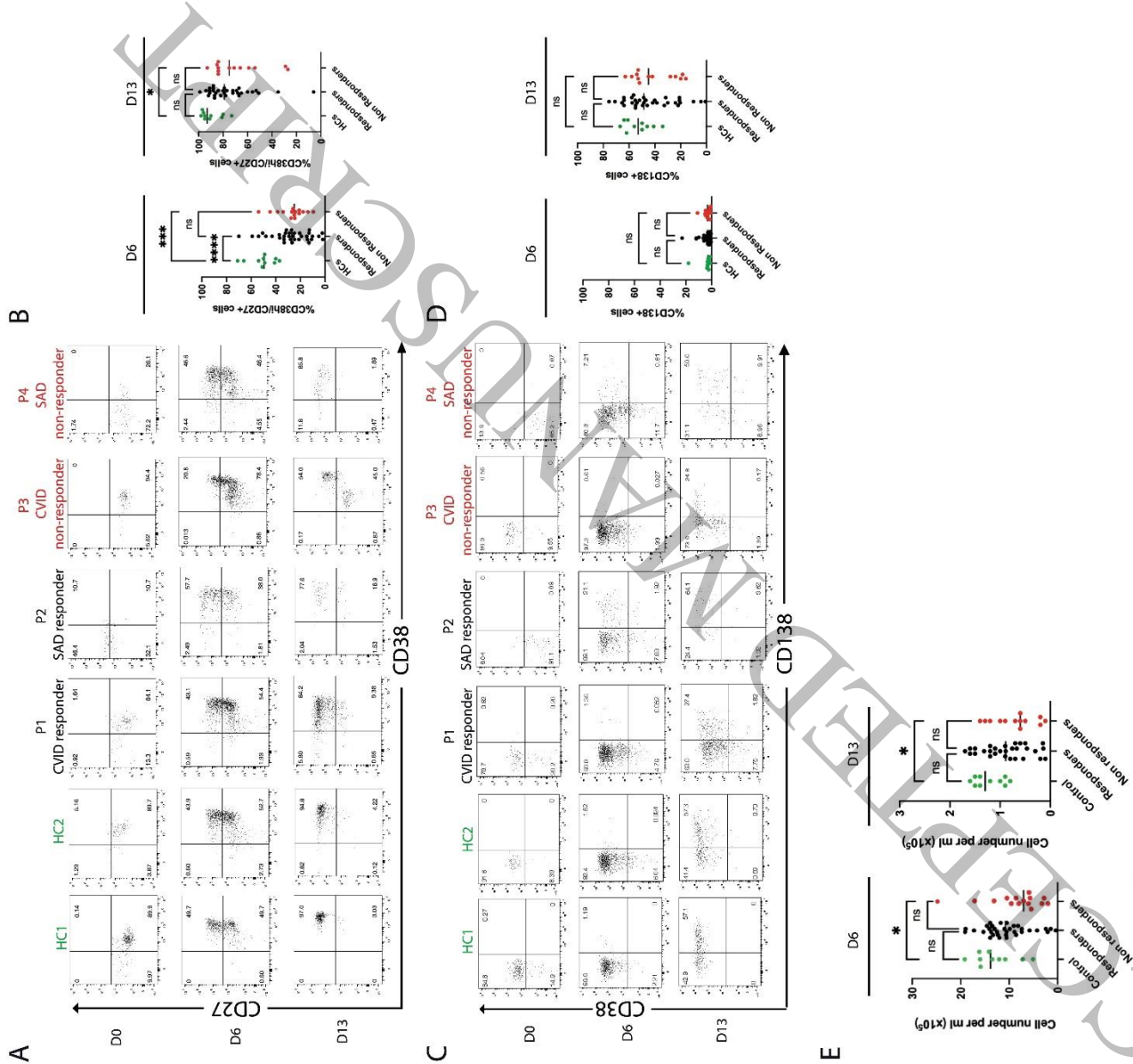


Figure 3

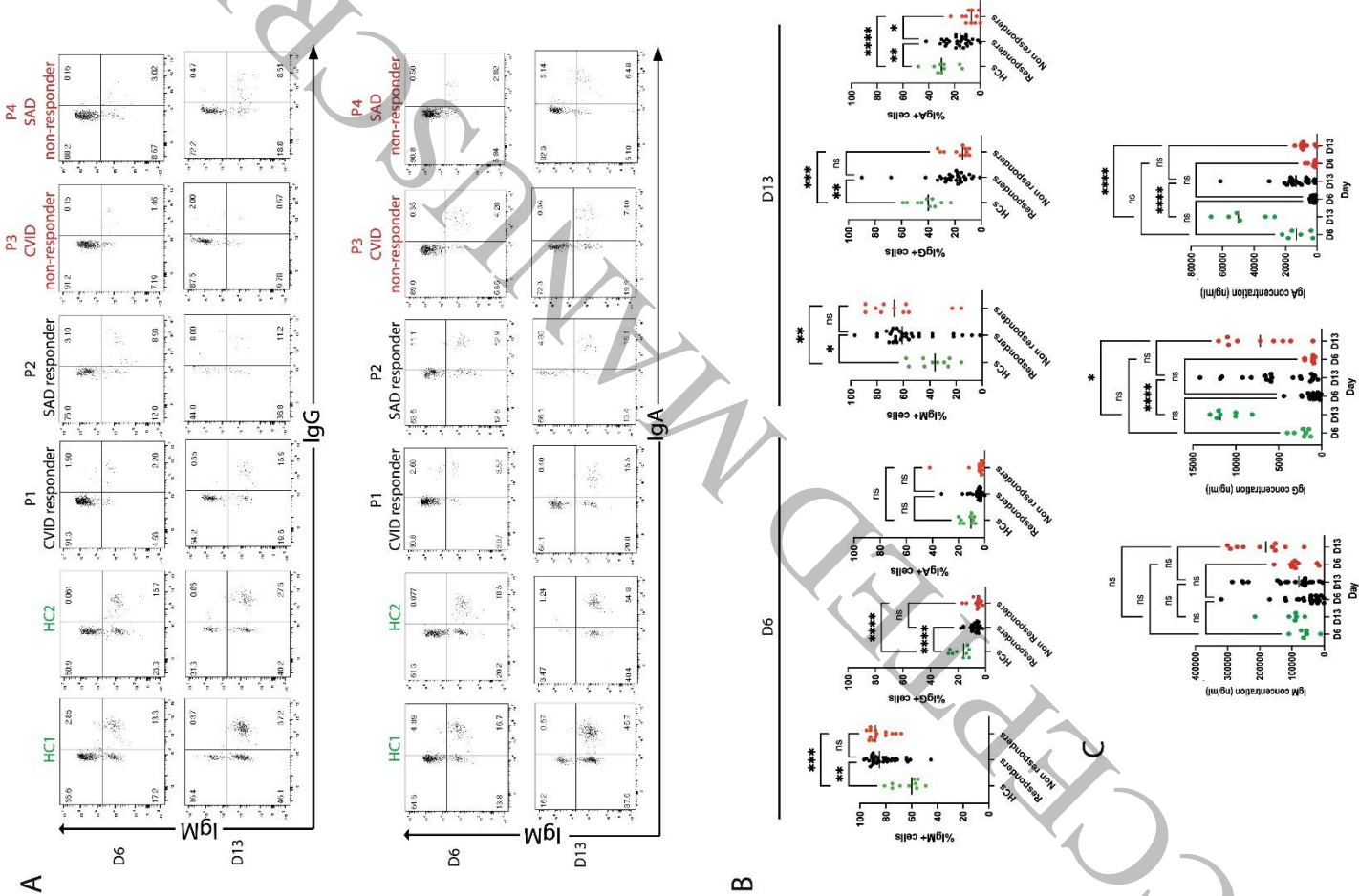


Figure 4

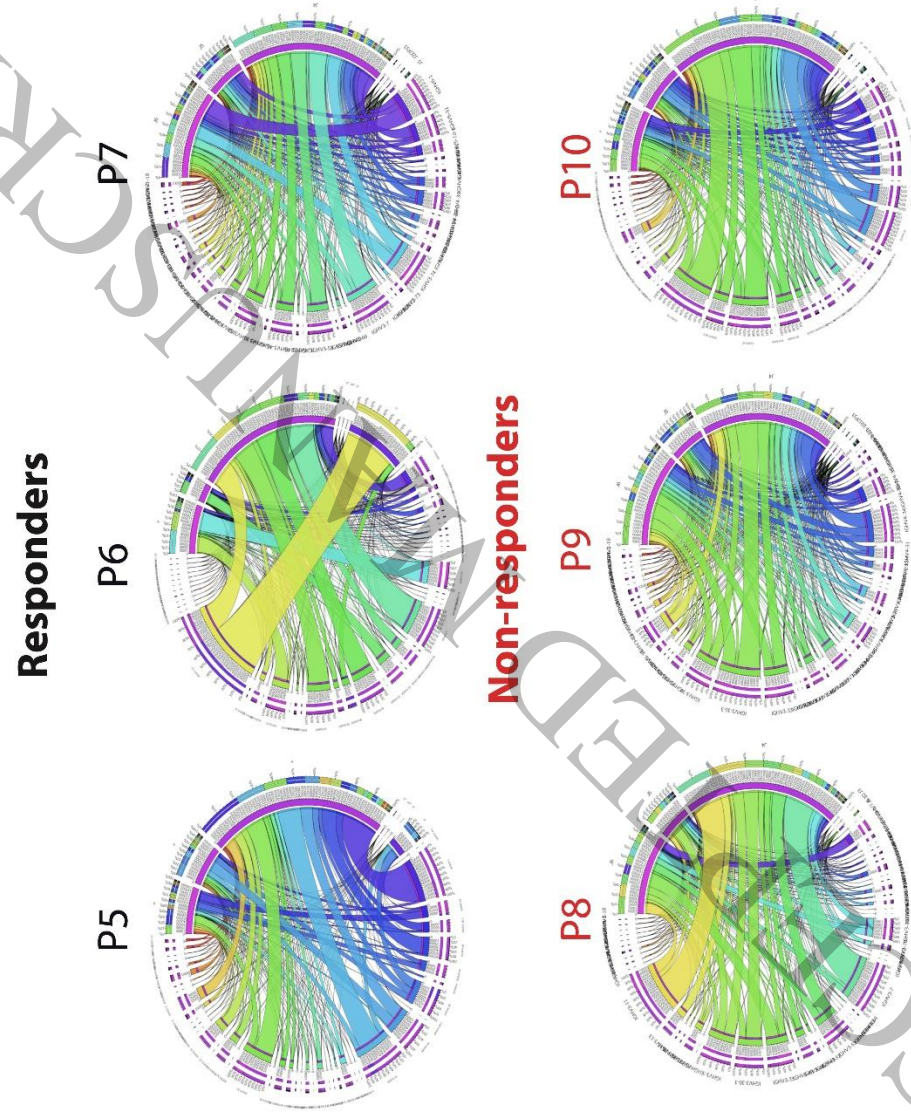


Figure 5

