JCI The Journal of Clinical Investigation

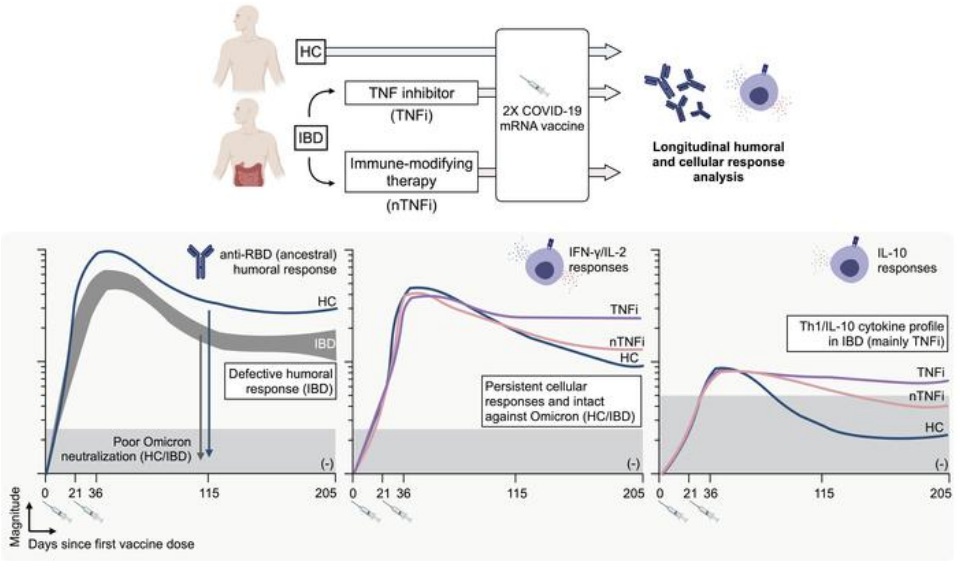
Favorable vaccine-induced SARS-CoV-2 specific T cell response profile in patients undergoing immune-modifying therapies

Martin Qui, Nina Le Bert, Webber Pak Wo Chan, Malcolm Tan, Shou Kit Hang, Smrithi Hariharaputran, Jean Xiang Ying Sim, Jenny Guek Hong Low, Weiling Ng, Wei Yee Wan, Tiing Leong Ang, Antonio Bertoletti, Ennaliza Salazar

J Clin Invest. 2022. https://doi.org/10.1172/JCI159500.

Clinical Research and Public Health | In-Press Preview | Immunology

Graphical abstract



Favorable vaccine-induced SARS-CoV-2 specific T cell response profile in patients undergoing immune-modifying therapies

Martin Qui[1], Nina Le Bert[1], Webber Pak Wo Chan[2], Malcolm Tan[2], Shou Kit Hang[1], Smrithi Hariharaputran[1], Jean Xiang Ying Sim[3], Jenny Guek Hong Low[1,3], Weiling Ng[4], Wei Yee Wan[4], Tiing Leong Ang[5], Antonio Bertoletti[1,6]*†, Ennaliza Salazar[2]†

[1]Programme in Emerging Infectious Diseases, Duke-NUS Medical School; Singapore.

[2]Department of Gastroenterology and Hepatology, Singapore General Hospital; Singapore.

[3]Department of Infectious Disease, Singapore General Hospital; Singapore.

[4]Department of Microbiology, Singapore General Hospital; Singapore.

[5]Department of Gastroenterology and Hepatology, Changi General Hospital; Singapore.

[6]Singapore Immunology Network, A*STAR; Singapore.

*Corresponding author. Address: Programme in Emerging Infectious Disease, Duke-NUS Medical School, 8 College Road, Singapore 169857, Singapore. Phone: +(65) 6601-2656; Email: antonio@duke-nus.edu.sg.

†These authors share co-senior authorship

Conflict of interest

N. Le Bert and A. Bertoletti reported a patent for a method to monitor SARS-CoV-2-specific T cells in biological samples pending. A. Bertoletti reported personal fees from Oxford Immunotech and Qiagen outside the submitted work. The other authors have declared that no other conflicts of interest exist.

ABSTRACT

BACKGROUND: Patients undergoing immune-modifying therapies demonstrate a reduced humoral response after COVID-19 vaccination, but we lack a proper evaluation of the impact of such therapies on vaccine-induced T cell responses.

METHODS: We longitudinally characterized humoral and Spike-specific T cell responses in inflammatory bowel disease (IBD) patients who are on antimetabolite therapy (azathioprine or methotrexate), TNF inhibitors and/or other biologic treatment (anti-integrin or anti-p40) for up to 6 months after completing two-dose COVID-19 mRNA vaccination.

RESULTS: We demonstrated that a Spike-specific T cell response is not only induced in treated IBD patients at levels similar to healthy individuals, but also sustained at higher magnitude for up to 6 months after vaccination, particularly in those treated with TNF inhibitor therapy. Furthermore, the Spike-specific T cell response in these patients is mainly preserved against mutations present in SARS-CoV-2 B.1.1.529 (Omicron) and characterized by a Th1/IL-10 cytokine profile.

CONCLUSION: Despite the humoral response defects, patients under immune-modifying therapies demonstrated a favorable profile of vaccine-induced T cell responses that might still provide a layer of COVID-19 protection.

FUNDING: This study was funded by the National Centre for Infectious Diseases NCID Catalyst Grant (FY2021ES) and the National Research Fund Competitive Research Programme (NRF-CRP25-2020-0003). The funders played no role in the design, conduct, or reporting of this study.

INTRODUCTION

Immune-modifying agents are the treatment of choice for different chronic inflammatory diseases of autoimmune origin. Antimetabolites (azathioprine and methotrexate) or biologics, such as TNF inhibitors (adalimumab and infliximab), anti-p40 (ustekinumab) or anti-integrin (vedolizumab and etrolizumab) antibodies, are used alone or in combination to reduce inflammatory events in the gut (i.e., Crohn's disease or ulcerative colitis), skin (i.e., psoriasis), joints (i.e., rheumatoid arthritis), or in multiple systems (i.e., systemic lupus erythematosus). While these agents reduce disease burden and improve quality of life (1), they are broadly considered immunosuppressive. The COVID-19 pandemic and the necessity to implement widespread vaccination sparked debate and research on the impact of these chronic therapies on the immunogenicity of SARS-CoV-2 vaccination (2–4).

Others have already shown that these therapies, particularly TNF inhibitors, reduce the ability of different COVID-19 vaccines (based on mRNA or Adenoviral vector) to produce Spike-specific antibodies (5–8), especially those that recognize SARS-CoV-2 variants including B.1.617.2 (Delta) (4). Such results are expected, since reduced humoral responses to other vaccines (i.e., anti-pneumococcal, anti-HBV) was already demonstrated in patients similarly undergoing TNF inhibitor or other antimetabolite therapy (9–11), and since TNF-alpha has been demonstrated to play an important role in the coordinate maturation of humoral immunity (12).

Nevertheless, antibodies can neither be considered the exclusive immunological parameter triggered by vaccination, nor the only determinant of its protective effect. Both mRNA- and adenoviral vector-based vaccines elicit humoral and cellular Spike-specific immunity (13–15) and an early induction of Spike-specific T cell responses

associate with the early protective effect of mRNA vaccination (16). In addition, the apparent indispensability of coordinated humoral and cellular immune activation for rapid and successful control of SARS-CoV-2 infection (17), and the rise to global circulation of the Omicron variant (18) both highlight the importance of the vaccine-induced T cell response. While Spike mutations have conferred the Omicron variant with the ability to evade the majority of vaccine-induced neutralizing antibodies (19), Spike-specific T cell immunity remains mainly intact against the Omicron variant (20–23).

While these T cells might not play a role in preventing infection, their ability to recognize and lyse virus-infected cells likely represents an important antiviral mechanism that might prevent the unchecked spread of SARS-CoV-2 in the infected host (24). However, the impact that different immune-modifying therapies exert on vaccine-induced Spike-specific cellular immunity has only started to be analyzed (25), with initial evidence of preserved cellular immunity levels at least immediately after vaccination.

In this manuscript, we therefore studied a cohort of patients with inflammatory bowel diseases (IBD) who are antimetabolites (AM), TNF inhibitor (TNFi) and/or other biologic treatment (anti-integrin and anti-p40), and we characterized both cellular and humoral vaccine-induced Spike-specific immunity. Spike-specific immune responses were analyzed from pre-vaccination, up until 6 months following the second dose of COVID-19 mRNA vaccination (BNT162b2 or mRNA-1273). Importantly, we designed an experimental plan to investigate not only the ability of vaccine to elicit “classical” Spike-specific T cell responses producing Th1 cytokines (IFN-γ and IL-2), but also the anti-inflammatory/regulatory IL-10 cytokine. The rationale of such an experimental

design was based on data demonstrating that TNFi therapy mediates induction of IL-10 in T cells that likely contribute to their ability to dampen inflammation (26, 27).

The ability to modify the functional profile of classical Th1 T cells can be of particular importance in SARS-CoV-2 infection. The presence and induction of both IL-10 and IFN-γ producing SARS-CoV-2 specific T cells is associated with asymptomatic SARS-CoV-2 infection (28) and hybrid immunity (29), while their absence has been reported in severe COVID-19 (30). The induction of such T cells endowed with anti-inflammatory potential might be advantageous in the asymptomatic control of SARS-CoV-2 infection. Furthermore, due to the pervasion of the Omicron variant globally, the ability of vaccine-induced Spike-specific T cells to tolerate the amino acid mutations characteristic of the Omicron variant necessitates evaluation. We therefore directly tested ex vivo the impact that Omicron variant mutations exert on the Spike-specific T cells induced in IBD patients under different treatments.

RESULTS

Study population

During the study period, 94 IBD patients had at least one blood sample processed for analysis (Figure 1). Forty-five patients completed at least three visits while the rest were either lost to follow up or recruited after first or second vaccination. Of the 94 patients, 63 (67%) patients had Crohn's disease and 31 (33%) had ulcerative colitis. Sixty-three (67%) IBD patients and 18 (36%) HC were male, while 31 (33%) IBD patients and 32 (64%) HCs were female (p=0.0004). Median ages (years) in IBD (39) and HC groups (41) were similar (p=1). All HCs and 93 (99%) IBD donors reported Asian ethnicity (1 White). Forty-nine (52%) patients were on TNFi, and 45 (48%) patients were on other non-TNFi (nTNFi) immunotherapy. Other baseline characteristics including age, gender, IBD diagnosis and duration, mRNA vaccine taken and steroid usage between TNFi and nTNFi groups were similar (Table 1). Among those undergoing TNF inhibitor therapy, 27 (55%) had an additional antimetabolite. Those on other nTNFi therapies consisted of 15 (33%) on an AM only, 7 (16%) on anti-p40 (a-p40) monotherapy and 11 (24%) on anti-integrin (a-Integrin) monotherapy. Those on an additional AM include 9 (20%) on a-p40 (a-p40+AM) and 3 (7%) on a-Integrin (a-Integrin+AM); those on a-Integrin+AM were excluded from analysis due to the lack of data points. Samples from 3 patients, all at the final timepoint (D 205), were collected but excluded from analysis due to COVID-19. Six patients were on concomitant steroid therapy: 2 in the TNFi group and 4 in the nTNFi group (p=0.6). Eight from the TNFi group and 7 patients from the nTNFi group took the mRNA-1273 (Moderna) vaccine (p=1). Further disease phenotype and behavior characteristics of the IBD cohort are shown in Table 2.

Vaccine-induced humoral immunity

SARS-CoV-2 Spike Receptor Binding Domain (RBD) IgG levels were quantified in response to COVID-19 mRNA vaccination both in the HCs and the IBD cohort (Figure 2). In line with previous observations (5–7), at all post-vaccination timepoints D 21 (just before second mRNA dose), D 36 (two weeks after two-dose vaccination), D 115 (three months after two-dose vaccination) and D 205 (six months after two-dose vaccination), the medians of IBD cohort humoral responses (geometric means/GMean of 176, 5658, 1006 and 310 AU/mL) were lower ($p<0.005$) than what was observed in HC (GMean of 1212, 14845, 2871 and 980 AU/mL) (Figure 2A).

The deficiency of vaccine-induced anti-RBD antibodies was evident in patients undergoing therapy with TNFi monotherapy on D 115 and even more in patients under combination therapy with TNFi and an antimetabolite (TNFi+AM; 3 and 6 months after two-dose vaccination) (Figure 2B). No significant differences among anti-RBD titers 3 and 6 months after vaccination (D 115 and D 205) in HC and IBD patients undergoing nTNFi therapies were observed. Patients undergoing AM, a-p40 and a-Integrin therapies on D 115 and D 205 displayed anti-RBD titers that were indistinguishable from HC controls. We also performed a surrogate viral neutralization test (sVNT) and found significantly reduced inhibition of Ancestral S-RBD binding to the human ACE2 receptor (hACE2) in TNFi+AM, TNFi and AM groups on D 115 (Figure 2C).

Vaccine-induced Spike-specific T cell responses

The magnitude and function of the Spike-specific T cell response induced by vaccination in HC and IBD patients was characterized directly in whole blood. A pool of 15-mer peptides covering the immunogenic regions of the SARS-CoV-2 S-protein (S pool) was used to measure Spike-specific T cell responses (Table S1). The quantity

of Th1 cytokines (IFN-γ and IL-2) secreted in the plasma after peptide stimulation was quantified after overnight incubation (Figure 3A). This rapid quantitative assay is demonstrated to possess identical sensitivities/specificities of conventional ELISpot assays (31).

Before vaccination, whole blood supernatants of HC and IBD patients stimulated with S pool displayed median IFN-γ and IL-2 levels below threshold. Some whole blood supernatants from either cohort exhibited cytokine production higher than unstimulated controls, consistent with the presence of Spike cross-reactive T cells already demonstrated in uninfected individuals (32, 33). Peptide-induced IFN-γ and/or IL-2 clearly increased in both HC and IBD patients after first (D 21) and second dose vaccination (D 36) in line with the ability of mRNA vaccines to induce Spike-specific T cells (13). In particular, 2 weeks after second vaccination, all HC (28/28 for both IFN-γ and IL-2) and the majority of IBD patients under immune-modifying therapies possessed positive IFN-γ (50/51) and IL-2 (49/51) responses (Figure 3B). Importantly, we did not observe any reduction of IFN-γ or IL-2 responses from 3 and 6 months after two-dose vaccination in IBD patients in comparison to HC.

We then analyzed cytokine levels induced in IBD patients under different treatment regimens. Longitudinal responses from individual donors are displayed in Figure 4A. As shown in Figure 4B, patients treated with TNFi alone demonstrated higher levels of IFN-γ responses than HC both at 3 and 6 months after two-dose vaccination. Although few, we also noted that patients treated with a-p40 biologics present high median levels of IL-2 production 3 months after two-dose vaccination (n=7, GMean 229.6 pg/mL). Importantly, no specific treatment caused an inhibition of the quantity of IFN-γ and IL-2 (D 115 and D 205) in comparison with HC; even the TNFi+AM group displaying lower Spike-specific humoral responses (Figure 2) and lower induction of T

cell responses on D 36 (Figure S1) demonstrated IFN-γ and IL-2 responses comparable to that in HC at these later timepoints.

We also compared the magnitude of Spike-specific T cell responses between IBD patients vaccinated with either BNT162b2 or mRNA-1273. No differences were observed in IFN-γ and IL-2 quantities at all post-vaccination timepoints, except an increased production of IL-2 in mRNA-1273 vaccinated IBD patients on D 115 and D 205 (Figure S2A). The finding of higher levels of IFN-γ responses in TNFi-treated IBD patients 3 and 6 months after two-dose vaccination is maintained when mRNA-1273 vaccinated donors are excluded from the analysis (Figure S2B-C).

Thus, mRNA vaccination in IBD patients undergoing treatment with different immune-modifying therapies demonstrated a Spike-specific T cell cytokine responses that is not inferior to what is detectable in HC. Furthermore, TNFi therapy was associated with a level of Spike-specific T cell responses 3 and 6 months after second vaccination (D 115 and D 205) greater than that of HC.

Spike-specific CD4+ and CD8+ T cells in vaccinated IBD patients and impact of Omicron variant mutations

To confirm that COVID-19 mRNA vaccination induces Spike-specific CD4+ and CD8+ T cells in IBD patients undergoing immune-modifying therapy, IBD patient PBMCs collected on D 115 were stimulated with a Spike peptide megapool (SP-MP) and analyzed for expression of activation induced markers (AIM) on gated CD4+ and CD8+ T cells (Figure 5A). Peptide stimulation activated more CD4+ than CD8+ T cells in all donor groups. Furthermore, while a lower frequency of AIM+ CD4+ was found in nTNFi donors than in HC, TNFi/TNFi+AM patients present frequencies of AIM+ CD4 and CD8+ T cells similar to HC.

The higher quantity of IFN-γ detected in peptide-stimulated whole blood of IBD donors on TNFi therapy could either be related to a cumulative increase in the cytokine secretion potential of individual Spike-specific T cells, or a larger fraction capable of IFN-γ secretion, rather than an increase in their frequency. Therefore, we quantified Spike specific CD4+ and CD8+ T cells able to produce IFN-γ and IL-2. Although low frequencies were detected, CD4+ IFN-γ+ T cells in TNFi treated IBD donors were enriched relative to HC, while geometric MFIs (GeoMFI) of either CD4+ or CD8+ IFN-γ among TNFi-treated donors and HC were similar (Figure 5B). Interestingly, IL-2+ CD4+ or CD8+ Spike-specific T cells were marginally higher in IBD patients than HC, reaching statistical significance for CD8+ T cells in TNFi+AM treated patients. Moreover, IL-2 GeoMFIs were significantly elevated in the Spike-specific CD4+ T cells of TNFi-treated donors and in CD8+ T cells of TNFi+AM and nTNFi-treated donors than in HC (Figure 5C). These findings hint that while vaccination under concurrent TNF inhibition induced similar amounts of antigen-specific T cells, HC and TNFi-treated IBD patients differed in IFN-γ and IL-2 producing fractions.

We then analyzed the impact of the mutations that characterize the Spike protein of the Omicron variant on the vaccine-induced Spike-specific T cells present in HC and in IBD patients (Figure 6). Patient PBMCs were stimulated with three peptide pools covering the entire Spike protein (253 peptides) of the ancestral SARS-CoV-2 (Table S2) and the regions mutated in the Omicron variant (67 peptides), with and without the amino acid-substitutions/deletions that characterize the SARS-CoV-2 Omicron variant (Table S3). We performed an IFN-γ ELISpot assay to quantify the frequency of SARS-CoV-2 specific T cells responding to conserved regions of the Spike protein, and to derive the frequency of responses altered by the variant-defining regions in the Omicron variant. As already seen in healthy vaccinated individuals (20, 21), the Spike-

specific T cell response to the Omicron variant was mainly preserved in all HC and IBD donors irrespective of their treatment. An inhibition of more than 25% of the total Spike-specific T cell response due to Omicron mutations was observed in only 1/12 HC and 1/14 IBD patient samples tested (Figure 6A). In contrast, pairwise analysis of neutralizing antibodies in D 115 donor sera by surrogate virus neutralization (sVNT) to both the ancestral and Omicron variant S-RBD demonstrated a dramatic decrease to below-threshold levels (<30% inhibition) in virtually all tested samples (49/50 HC and 63/63 IBD) for hACE2-RBD binding inhibition (Figure 6B).

Immune-modifying therapies increase IL-10 production of Spike-specific T cells

Differences in the kinetics of Spike peptide-induced IFN-γ and IL-2 detected in IBD patients undergoing TNFi therapy suggest that this treatment might modify vaccine-induced Spike-specific T cells. In addition, TNFi therapy has been shown to modify T cell function through expression of a transcriptional signature that upregulates IL-10 production in T cells (27). We therefore tested whether cytokine secretion profiles in whole blood supernatants after Spike-peptide stimulation contains not only classical Th1 cytokines IFN-γ and IL-2, but also IL-10. The quantity of IL-10 detected in IBD treated patients and HC before and after two dose vaccination was measured (Figure 7).

At both timepoints following first (D 21) and second dose vaccination (D 36), increased concentrations of IL-10 were detected in whole blood supernatants of HC and IBD patients relative to their respective pre-vaccination baselines. Furthermore, at 3 and 6 months after the second vaccine dose (D 115 and D 205), no IL-10 was detected in the majority of HC, while we noticed a persistence of IL-10 induction in IBD patients (Figure 7A). Values of quantified IL-10 (e.g., 9.65 pg/mL on D 115) were low in comparison to corresponding IL-2 (61.5 pg/mL) and IFN-γ (31.5 pg/mL) responses in

IBD patients. Particularly, sustained production of IL-10 in peptide-stimulated whole blood was observed in both TNFi-treated subcohorts both at 3- and 6-months after two-dose vaccination (Figure 7B).

To characterize the chronological evolution of cytokine profiles, we used UMAP to integrate quantified, log-transformed IL-10 data with IFN-γ and IL-2 for each donor-timepoint (Figure 7C). UMAP projections of datapoints originating from pre-vaccination samples of either HC or IBD patients formed a distinct cluster. Moreover, the datapoints co-segregated following first dose (D 21) and two weeks after second dose vaccination (D 36), further highlighting the similarities of cellular responses between the two cohorts. Notably, 3 months after completion of the two-dose regimen (D 115), the cytokine profiles diverged into distinct clusters, with IBD patient profiles coinciding with regions defined by higher IL-10, IFN-γ and IL-2. This observed clustering persisted up to 6 months after two-dose vaccination. Further analysis showed that IL-10 production did not correlate with either IFN-γ or IL-2 in HC, while a significant but weak correlation existed between IFN-γ and IL-10 two weeks (D 36) and 3 months (D 115) after the second dose in IBD patients (Figure S3).

To then confirm that Spike peptide pool stimulation induces IL-10 production in T cells, we performed direct ex vivo intracellular staining of donor PBMCs on 3-month (D 115) samples. Indeed, the low magnitude of cumulative IL-10 we observed in whole blood stimulated supernatants implied the identification of IL-10+ T cells to be a technically challenging feat. Intracellular cytokine staining revealed IL-10 accumulation upon Spike peptide pool stimulation mainly detected in CD4+ T cells (Figure S4, A and B). Furthermore, only IL-10+ CD4+ T cells were enriched in PBMCs of IBD patients on TNFi/TNFi+AM (TNFi±AM) (Figure 7D and S4C). None of the samples demonstrated distinct populations of T cells co-staining for both IFN-γ and IL-10 intracellularly (Figure

S4, D and E), suggesting that production of these cytokines may occur independently or that the method utilized is unsuitable for co-staining analysis.

DISCUSSION

The attenuated humoral responses detected in SARS-CoV-2 vaccinated patients under different immune-modifying treatments, particularly in those treated with TNFi therapy, have generally been interpreted to imply reduced vaccine immunogenicity, fueling debate on the possible increased risk of severe COVID-19 in patients treated chronically with these immune-modifying therapies (2, 3, 6, 7). Here, by studying IBD patients under various regimens and vaccinated with the prevailing Spike-based mRNA vaccines, we demonstrated that a Spike-specific T cell response is not only induced in IBD treated patients to levels similar to that in HC, but also persisted longer and at higher levels particularly in the ones treated with TNFi.

In contrast to the role of antibodies, T cells cannot prevent infection; instead, they excel in the clearance of intracellular pathogens either through recognition and lysis of infected cells or activating macrophages, and support B cell maturation (32). Furthermore, since coordination between humoral and cellular arms of immunity is likely to be essential for rapid viral control and reduced pathogenicity (17), we cannot claim that the increased T cell immunogenicity observed directly translates into better protective efficacy of vaccination in patients under immune-modifying therapies. Nevertheless, these patients, particularly those undergoing TNFi therapy, were clearly able to mount a robust Spike-specific cellular immunity. Additionally, TNFi therapy did not abolish, but only reduced production of antibodies after mRNA vaccination. Previous observations (5-8) and our own data demonstrate this. It is possible therefore to hypothesize that the presence of cellular immunity against Spike compensates for the observed humoral defect.

In this aspect, the demonstration provided here that vaccine-induced Spike-specific T cells of IBD patients are minimally altered in their ability to recognize Omicron variant Spike adds a further layer of reassurance. Several recent works have shown that vaccine-induced Spike-specific T cells in healthy individuals is mainly preserved against the Omicron variant (20–23). Our data in IBD patients under different immune-modifying treatments demonstrated a similar pattern of reduced recognition only in a minority of tested patient samples. This finding suggests that, as in healthy vaccinated individuals (20, 21), the Spike-specific T cells of patients undergoing various immune-modifying regimens mount a multispecific T cell response against different conserved regions of Spike. Therefore, vaccinated patients undergoing TNFi therapy may develop reliable protection against severe disease. By analyzing the kinetics of the Spike-specific T cell response, we observed that the higher levels of IFN-γ secretion present 3 and 6 months after vaccination in TNFi treated patients, in comparison to HC, did not derive from a higher level of vaccine-induced Spike-specific T cell induction at earlier time points, rather more likely from a propensity of the T cell response to persist longer. Our findings can be explained by the differential effect of TNF-alpha on humoral and cellular immune responses. While TNF-alpha downregulates T cell expansion (34), it supports B cell maturation (12). Therefore, in the context of vaccination, TNF-alpha inhibition can cause a reduction of subsequent B cell maturation with reduced antibody quantities (12), but a persistence of vaccine-induced T cells (34).

The inhibition of TNF-alpha, directly through TNFi or indirectly through other immunomodulatory treatments, can also explain the simultaneous induction of IFN-γ, IL-2 and IL-10 found in IBD patients under different treatments. Blockade of the effect of TNF-alpha on T cells with TNF-alpha inhibitors is known to upregulate IL-10 in T

cells (27). The presence of Spike-specific Th1 and IL-10 producing cells can be advantageous in SARS-CoV-2 infection. Animal models have shown that the ability of T cells to secrete IFN-γ and IL-10 simultaneously led to effective viral control without triggering severe pathological processes (35–37). Previously, we also observed that a pattern of cytokine production characterized by the simultaneous presence of IFN-γ, IL-2 and IL-10 constitute the T cell response detected in patients who control SARS-CoV-2 infection asymptomatically (28). The importance of IL-10 and IFN-γ production by T cells has also been highlighted by two recent works: such a functional T cell profile was demonstrated to be defective in severe COVID-19 (30), while the presence of IL-10 producing Spike-specific T cells is characteristic of individuals with hybrid SARS-CoV-2 immunity (29) who demonstrate a robust immunity from re-infection (38, 39). Regardless of our inability to visualize T cells co-expressing IL-10 and IFN-γ by ICS, the aggregate production of IL-10 and IFN-γ, particularly in TNFi-treated donors, may deliver functionally similar outcomes. Of note, the demonstration that mRNA vaccination in IBD patients undergoing TNF inhibition resulted in the induction of T cells with an IFN-γ/IL-2/IL-10 secretion profile suggests that similar functional profiles might likewise be induced in virus-specific T cells after SARS-CoV-2 infection, explaining the clinical observation that SARS-CoV-2 infection in patients undergoing TNFi treatment is generally mild (40–42). In our own study, 6 patients who eventually developed COVID-19 all had a mild disease course and did not require hospitalization; 4 of whom were on TNFi.

There are some limitations in this study: the most important one being that the bulk of T cell experiments were performed not by direct measurement of T cell quantity, but by measuring cytokines secreted in whole blood after specific peptide stimulation. However, we provided direct evidence orthogonally by demonstrating Spike-specific

CD4+ and CD8+ T cells induced by vaccination in IBD patients and visualizing IL-10+, IFN-γ+ and IL-2+ T cells upon Spike peptide stimulation. Nevertheless, while whole blood cytokine release does not directly quantify the number of antigen-specific T cells, it provides a standardized method well suited for longitudinal analysis of T cell responses in patients under different treatments. The simplicity of the assay reduces the inter-assay variability, and is directly performed on fresh whole blood, limiting the detrimental effects of freezing and thawing (43). Furthermore, since T cell functionality is analyzed in whole blood, the immune-modifying therapies administered into the patients are present at therapeutic levels during the assay, mimicking, as we previously argued (44), more closely the situation in vivo.

In conclusion, we have shown here that mRNA vaccination in IBD patients under different immunomodulatory treatments triggered a robust cellular immune response amidst an attenuated humoral response. Particularly, patients under TNFi monotherapy demonstrated reduced kinetics of decline of Spike-specific T cell responses, and an ability to secrete a cytokine profile characterized by the simultaneous presence of IFN-γ, IL-2 and the anti-inflammatory IL-10 cytokine upon Spike encounter. Since this T cell functional profile has been preferentially associated with asymptomatic SARS-CoV-2 control (28), COVID-19 mRNA vaccination in individuals under such immunomodulatory therapies might still offer a layer of protection. Moreover, these may even offer some advantages in controlling SARS-CoV-2 infection with limited pathological sequelae.

METHODS

Study design

This is a prospective, observational study conducted to assess both humoral and cellular responses to mRNA-based COVID-19 vaccines (BNT162b2 and mRNA-1273) in IBD patients who were treated with antimetabolites, TNFi and/or other biologics from July 2021 to February 2022. Specifically, the included therapies were azathioprine or methotrexate for antimetabolites, adalimumab or infliximab for TNFi, ustekinumab for anti-p40, and vedolizumab or etrolizumab for anti-integrin. Patients have completed two same-dose vaccine courses three weeks apart with either one of the COVID-19 mRNA vaccines (n=94). The HC group included healthcare professionals not undergoing immune-modifying therapy (n=50). Patients younger than 18 years old, those with previous SARS-CoV-2 polymerase chain reaction-confirmed COVID-19, or pregnant women were all excluded. Demographic data were self-reported based on their national registry classification. Samples were collected at baseline pre-vaccination (D 0), three weeks (D 21± 5 days) after first dose of vaccine, 2 weeks (D 36 ± 5 days) after second dose of vaccine, 3 months (D 115 ± 5 days) after second dose of vaccine and 6 months (D 205 ± 5 days) after second dose of vaccine. Due to the rapidity of vaccination uptake, recruitment of patients before vaccination became more challenging. Hence the protocol was extended to include IBD patients who were on antimetabolites/biologics and received their first and/or second dose of vaccine to be followed up longitudinally according to the study interval for blood sampling.

Quantification of humoral responses

Measurements were performed using the Abbott Architect i2000 automated analyzer using the SARS-CoV-2 IgG II Quant assay, a chemiluminescent microparticle

immunoassay (CMIA) for the quantitative detection of IgG targeting the receptor binding domain (RBD) of the S1 subunit of the spike protein of SARS-CoV-2. Results are expressed as AU/mL, where values ≥50.0 AU/mL are interpreted as positive.

Surrogate virus neutralization test

Inhibition rates of hACE2 receptor binding to S-RBD by neutralizing antibodies were derived using the GenScript SARS-CoV-2 Surrogate Virus Neutralization Test (sVNT) kit according to the manufacturer's protocol and equation, with the ancestral and Omicron variant SARS-CoV-2 S-RBD. Patient sera were diluted 1:19 with sample dilution buffer and combined 1:1 with either ancestral SARS-CoV-2 HRP-RBD or Omicron variant HRP-RBD for 30 minutes at 37°C. The resulting mixture was added onto the hACE2 receptor-coated capture plate and incubated for 15 minutes at 37°C. The plate was washed four times before incubation in TMB solution. The reaction was stopped with Stop Solution, and the solutions were read at 450nm in a microtiter plate reader (Tecan Spark 10M). A negative result was called for values <30% signal inhibition.

Quantification of cellular responses and analysis

We used a cytokine release assay (CRA) of whole peripheral blood stimulated using a SARS-CoV-2 spike-derived peptide (S) pool (Table S1) described previously (31). Freshly drawn whole blood (320µL; within 6 hours of venipuncture) was mixed with 80µL RPMI and stimulated with S pool peptides to a final peptide concentration of 2µg/mL or mixed with an equivalent amount of DMSO as control. Culture supernatants were collected 16 hours after culture and stored at −80°C until cytokine quantification. IFN-γ/IL-2 or IL-10 concentrations in plasma were quantified using an Ella machine (ProteinSimple) with microfluidic multiplex cartridges following the manufacturer's

instructions. Background cytokine levels quantified from DMSO controls were subtracted from the corresponding peptide pool stimulated samples. The threshold for a positive response was set at 10 times the lower limit of quantification for each cytokine (IFN-γ = 1.7 pg/mL; IL-2 = 5.4 pg/mL; IL-10 = 5.8 pg/mL). A pseudocount of 1 pg/mL was applied to the dataset for logistic transformation. Subsequently, log-transformed concentrations of each cytokine in all culture supernatants were projected onto UMAP space using 15 nearest neighbors (nn), min_dist of 0.2 and Euclidean distance.

Peripheral blood mononuclear cell separation

Peripheral blood mononuclear cells (PBMC) from HBSS-diluted anticoagulated blood (1:1) were separated by Ficoll-Paque density gradient centrifugation. PBMCs were frozen in FBS containing 10% DMSO and stored in liquid nitrogen until use.

ELISpot assay

ELISpot plates (Millipore) were coated with human IFN-γ antibody overnight at 4°C. Cryopreserved PBMCs were thawed and seeded at a density of 400,000 cells per well and stimulated with a respective peptide pool for 18 hours (2μg/mL) or an equivalent amount of DMSO (negative control). The plates were then incubated with human biotinylated IFN-γ detection antibody, followed by Streptavidin-AP, and developed using the KPL BCIP/NBT Phosphatase Substrate. To quantify positive peptide-specific responses, twice the number of mean spots of the unstimulated wells were subtracted from the peptide-stimulated wells, and the results expressed as spot forming cells (SFU)/10^6 PBMC. Results were excluded if negative control wells had >30 SFU/10^6 PBMC or positive control wells (PMA/Ionomycin) were negative.

Measurement of the impact of Omicron variant on total Spike-specific T cells

We directly tested donor PBMCs by IFN-γ ELISpot for reactivity against the ancestral or Omicron variant Spike protein. To quantify total responses to the ancestral Spike, we used a 10-amino acid overlapping 15-mer peptide pool (SP-MP) covering the entire Spike protein listed in Table S2 (1273 amino acids). For Omicron variant Spike responses, we designed two peptide pools (Table S3): one consisting of ancestral-derived Spike peptides covering the variable regions (termed the "Spike Hotspot-Ancestral" pool) and another consisting of the Omicron-derived Spike peptides covering the same region (termed the "Spike Hotspot-Omicron" pool). From this, we derived the total spot forming units (SFU) formed against the entire Omicron variant Spike using the equation below:

$$\mathrm{SFU}_{\text{Total Omicron Spike}} = \mathrm{SFU}_{\text{SP-MP}} - \mathrm{SFU}_{\text{Spike Hotspot-Ancestral}} + \mathrm{SFU}_{\text{Spike Hotspot-Omicron}}$$

From this, % inhibition due to variation in Omicron variant Spike sequences may be quantified using the equation below:

$$\text{Inhibition} = \frac{\mathrm{SFU}_{\text{SP-MP}} - \mathrm{SFU}_{\text{Total Omicron-Spike}}}{\mathrm{SFU}_{\text{SP-MP}}}$$

Activation-induced marker (AIM) assay

For each condition, 1 million PBMCs in 150µL AIM-V + 2% AB were stimulated for 24 hr at 37°C with a megapool (2µg/mL) of 15-mer peptides encompassing the full spike protein (SP-MP) or an equivalent amount of DMSO in the presence of 1µg/mL CD28/CD49d co-stimulation (BD). Cells were then washed in FACS buffer with 2mM EDTA, then stained with surface markers (CD3, CD4, CD8, CD69, CD134 and CD137 mAbs) diluted in FACS buffer (RT for 25 minutes). Dead cells were excluded using the Fixable Yellow Live/Dead fixable cell stain kit (Invitrogen). After 2 more washes in

FACS buffer, the cells were resuspended in PBS + 1% FA prior to analysis. The gating strategy is outlined in Figure S5, and the staining reagents used are outlined in Table S4. Reported frequencies of AIM+ cells are background-subtracted from DMSO/unstimulated samples, with a pseudocount of 10^{-4} added to represent below-background or null (0) frequencies in log-scale.

Intracellular cytokine staining

For each condition, 1 million PBMCs in 150µL AIM-V + 2% AB were stimulated for 24 hr at 37°C with SP-MP (2µg/mL) or an equivalent amount of DMSO in the presence of 1µg/mL CD28/CD49d co-stimulation (BD). In the last 4 hours, 1µg/mL Brefeldin A and 0.5X Monensin (Biolegend) were added. Cells were then washed in FACS buffer containing 2mM EDTA, then stained with surface markers (CD3, CD4 and CD8 mAbs) diluted in FACS buffer (RT for 25 minutes). Dead cells were excluded using the Fixable Yellow Live/Dead fixable cell stain kit (Invitrogen). Cells were washed twice in FACS buffer and fixed in Cytofix/Cytoperm (BD) for 20 minutes on ice. Cells were then washed with Perm/Wash (BD) solution prior to intracellular staining (anti-IFN-γ, anti-IL-2 or anti-IL-10). After 2 more washes in FACS buffer, the cells were resuspended in PBS + 1% FA prior to analysis. Similar to above, the gating strategy is outlined in Figure S5, and the staining reagents used are outlined in Table S4**.** Reported frequencies of cells staining for cytokines are background-subtracted from DMSO/unstimulated samples, with a pseudocount of 10^{-5} added to represent below-background (0) or null frequencies in log-scale. GeoMFIs reported for positive-staining populations are subtracted from negative-staining population GeoMFIs.

Flow cytometry

All flow cytometry samples were analyzed using cryopreserved cells which were thawed and resuspended in AIM-V media supplemented with 2% AB serum. Samples were stained accordingly and fixed in PBS + 1% FA. Acquisition was performed on a BD-LSR II Analyzer (BD) or CytoFLEX S (Beckman Coulter) within 24 hours and analyzed with FlowJo (BD)

Statistics and data analysis

Statistical analyses were performed using R Statistical Software (version 4.0.3) (ggpubr::stat_compare_means) and GraphPad Prism 9. For analysis of the study population, Wilcoxon signed rank test and Chi-square tests were used as indicated. Median values in each group for humoral, cellular, IL-10 and T cell subset analysis were compared by Kruskal-Wallis test (with Dunn's post-hoc multiple comparison test) or Wilcoxon signed rank test. For pairwise analysis, Wilcoxon matched-pairs signed rank test was performed. For correlation analysis, Spearman's rank correlation coefficient (ρ) was calculated. Where applicable, statistical tests used and the definitions are indicated in the figure legends. P values <0.05 were considered statistically significant. Categorized data with less than 3 independent samples were not included for statistical analysis. Data from flow cytometry was analyzed using FlowJo (BD).

Study approval

The study protocol was reviewed and approved by Institutional Research Board of Singhealth (IRB no: 2021/2398). All donors provided written consent for enrolment.

AUTHOR CONTRIBUTIONS

MQ, NLB, AB and ES conceptualized and designed the experiments. MQ, HSK, WYW and WLN performed experiments for measuring humoral responses. MQ, HSK and SH performed experiments for measuring cellular responses. MQ, NLB, AB and ES analyzed the data. MQ and ES prepared the figures and tables. SXYJ, JGHL and ES acquired funding for the project. WPWC, MT, ES, JGHL and TLA collected donor samples. Writing was prepared and edited by MQ, NLB, WYW, AB and ES.

ACKNOWLEDGEMENTS

We would like to acknowledge the contribution of SGH-IBD team members Dr Thomson Lim Chong Teik (MD), Dr Tay Shu Wen (MD), Loy Kia Lan (Nurse practitioner), Lim Teong Guan (Pharmacist), Dr Ong Wan Chee (Pharmacist), Dr Valerie Ng Yun Ting (Pharmacist) and Amanda Wong Shi Yi (Pharmacist) who helped in recruiting patients. We also would like to thank Abigail Yeong, Tan Hui Fang and Stephanie Ren for coordinating the logistics of the study and follow-ups for all the study participants. We would like to thank Ruklanthi de Alwis and Tanamas Siriphanitchakorn for their help with the neutralization assay. We would like to thank the AB lab members for their insights and critique. Lastly, we would like to thank all our patients who took their time off to participate in the study. This study was supported by funds under the NCID Catalyst Grant administered by the National Centre for Infectious Diseases (FY2021ES) and by the National Research Fund Competitive Research Programme (NRF-CRP25-2020-0003). Any opinions, findings and conclusions or recommendations expressed in this material are those of the author(s) and do not reflect the views of NCID.

REFERENCES

1. Turner D et al. STRIDE-II: An Update on the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) Initiative of the International Organization for the Study of IBD (IOIBD): Determining Therapeutic Goals for Treat-to-Target strategies in IBD. *Gastroenterology* 2021;160(5):1570–1583.

2. Alexander JL et al. SARS-CoV-2 vaccination for patients with inflammatory bowel disease: a British Society of Gastroenterology Inflammatory Bowel Disease section and IBD Clinical Research Group position statement. *Lancet Gastroenterology Hepatology* 2021;6(3):218–224.

3. Siegel CA et al. SARS-CoV-2 vaccination for patients with inflammatory bowel diseases: recommendations from an international consensus meeting. *Gut* 2021;70(4):635–640.

4. Chen RE et al. Reduced antibody activity against SARS-CoV-2 B.1.617.2 delta virus in serum of mRNA-vaccinated individuals receiving tumor necrosis factor-α inhibitors. *Med* 2021;2(12):1327-1341.e4.

5. Kennedy NA et al. Infliximab is associated with attenuated immunogenicity to BNT162b2 and ChAdOx1 nCoV-19 SARS-CoV-2 vaccines in patients with IBD. *Gut* 2021;70(10):1884–1893.

6. Edelman-Klapper H et al. Lower Serologic Response to COVID-19 mRNA Vaccine in Patients With Inflammatory Bowel Diseases Treated With Anti-TNFα. *Gastroenterology* 2022;162(2):454–467.

7. Wong S-Y et al. Serologic Response to Messenger RNA Coronavirus Disease 2019 Vaccines in Inflammatory Bowel Disease Patients Receiving Biologic Therapies. *Gastroenterology* 2021;161(2):715-718.e4.

8. Alexander JL et al. COVID-19 vaccine-induced antibody responses in immunosuppressed patients with inflammatory bowel disease (VIP): a multicentre, prospective, case-control study. *Lancet Gastroenterology Hepatology* 2022; doi:10.1016/s2468-1253(22)00005-x.

9. Salinas GF et al. Anti-TNF treatment blocks the induction of T cell-dependent humoral responses. *Ann Rheum Dis* 2013;72(6):1037.

10. Pratt PK et al. Antibody Response to Hepatitis B Virus Vaccine is Impaired in Patients With Inflammatory Bowel Disease on Infliximab Therapy. *Inflamm Bowel Dis* 2018;24(2):380–386.

11. Fiorino G et al. Effect of Immunosuppression on Immune Response to Pneumococcal Vaccine in Inflammatory Bowel Disease: A Prospective Study. *Gastroenterology* 2011;140(5):S-158.

12. Pasparakis M, Alexopoulou L, Episkopou V, Kollias G. Immune and inflammatory responses in TNF alpha-deficient mice: a critical requirement for TNF alpha in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response. *J Exp Medicine* 1996;184(4):1397–1411.

13. Sahin U et al. BNT162b2 vaccine induces neutralizing antibodies and poly-specific T cells in humans. *Nature* 2021;595(7868):572–577.

14. Khoo NKH et al. Differential immunogenicity of homologous versus heterologous boost in Ad26.COV2.S vaccine recipients. *Med* 2022; doi:10.1016/j.medj.2021.12.004.

15. Alter G et al. Immunogenicity of Ad26.COV2.S vaccine against SARS-CoV-2 variants in humans. *Nature* 2021;596(7871):268–272.

16. Kalimuddin S et al. Early T cell and binding antibody responses are associated with Covid-19 RNA vaccine efficacy onset. *Med* 2021;2(6):682-688.e4.

17. Moderbacher CR et al. Antigen-Specific Adaptive Immunity to SARS-CoV-2 in Acute COVID-19 and Associations with Age and Disease Severity. *Cell* 2020;183(4):996-1012.e19.

18. Viana R et al. Rapid epidemic expansion of the SARS-CoV-2 Omicron variant in southern Africa. *Nature* 2022 ; doi:10.1038/d41586-021-03832-5

19. Cao Y et al. Omicron escapes the majority of existing SARS-CoV-2 neutralizing antibodies. *Nature* 2021 ; doi:10.1038/d41586-021-03796-6

20. Gao Y et al. Ancestral SARS-CoV-2-specific T cells cross-recognize Omicron. *Nat Med* 2022 ; doi:10.1038/d41591-022-00017-z.

21. Tarke A et al. SARS-CoV-2 vaccination induces immunological T cell memory able to cross-recognize variants from Alpha to Omicron. *Cell* 2022;185(5):847-859.e11.

22. Liu J et al. Vaccines Elicit Highly Conserved Cellular Immunity to SARS-CoV-2 Omicron. *Nature* 1–7 (2022) doi:10.1038/s41586-022-04465-y.

23. Naranbhai V et al. T cell reactivity to the SARS-CoV-2 Omicron variant is preserved in most but not all individuals. *Cell* 2022;185(7):1259.

24. Bertoletti A, Tan AT, Le Bert N. The T-cell response to SARS-CoV-2: kinetic and quantitative aspects and the case for their protective role. *Oxf Open Immunol* 2021;2(1):iqab006.

25. Reuken PA et al. T cell response after SARS-CoV-2 vaccination in immunocompromised patients with inflammatory bowel disease. *J Crohn's Colitis* 2021;jjab147.

26. Roberts CA, Durham LE, Fleskens V, Evans HG, Taams LS. TNF Blockade Maintains an IL-10+ Phenotype in Human Effector CD4+ and CD8+ T Cells. *Front Immunol* 2017;8:157.

27. Evans HG et al. TNF-α blockade induces IL-10 expression in human CD4+ T cells.. *Nat Commun* 2014;5(1):3199.

28. Le Bert N et al. Highly functional virus-specific cellular immune response in asymptomatic SARS-CoV-2 infection. *J Exp Med* 2021;218(5):e20202617.

29. Rodda LB et al. Imprinted SARS-CoV-2-specific memory lymphocytes define hybrid immunity. *Cell* 2022; doi:10.1016/j.cell.2022.03.018.

30. Chauss D et al. Autocrine vitamin D signaling switches off pro-inflammatory programs of TH1 cells. *Nat Immunol* 2021;23(1):62–74.

31. Tan AT et al. Rapid measurement of SARS-CoV-2 spike T cells in whole blood from vaccinated and naturally infected individuals. *J Clin Invest* 2021;131(17). doi:10.1172/jci152379

32. Braun J et al. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. *Nature* 2020;587(7833):270–274.

33. Mateus J et al. Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans. *Science* 2020;eabd3871.

34. Suresh M, Singh A, Fischer C. Role of tumor necrosis factor receptors in regulating CD8 T-cell responses during acute lymphocytic choriomeningitis virus infection. *J Virol* 2005;79(1):202–13.

35. Zhao J et al. Airway Memory CD4 + T Cells Mediate Protective Immunity against Emerging Respiratory Coronaviruses. *Immunity* 2016;44(6):1379–1391.

36. Zhuang Z et al. Mapping and role of T cell response in SARS-CoV-2–infected mice. *J Exp Med* 2021;218(4):e20202187.

37. Sun J, Madan R, Karp CL, Braciale TJ. Effector T cells control lung inflammation during acute influenza virus infection by producing IL-10. *Nat Med* 2009;15(3):277–284.

38. Reynolds CJ et al. Prior SARS-CoV-2 infection rescues B and T cell responses to variants after first vaccine dose. *Science* 2021;372(6549):1418–1423.

39. Abu-Raddad LJ et al. Association of Prior SARS-CoV-2 Infection With Risk of Breakthrough Infection Following mRNA Vaccination in Qatar. *Jama* 2021;326(19):1930.

40. Salesi M, Shojaie B, Farajzadegan Z, Salesi N, Mohammadi E. TNF-α Blockers Showed Prophylactic Effects in Preventing COVID-19 in Patients with Rheumatoid Arthritis and Seronegative Spondyloarthropathies: A Case–Control Study. *Rheumatology Ther* 2021;8(3):1355–1370.

41. Haberman R et al. Covid-19 in Immune-Mediated Inflammatory Diseases — Case Series from New York. *New Engl J Medicine* 2020;383(1):NEJMc2009567.

42. Chappell H et al. Immunocompromised children and young people are at no increased risk of severe COVID-19. *J Infect* 2021;84(1):31–39.

43. Ford T et al. Cryopreservation-related loss of antigen-specific IFNγ producing CD4+ T-cells can skew immunogenicity data in vaccine trials: Lessons from a malaria vaccine trial substudy. *Vaccine* 2017;35(15):1898–1906.

44. Tan AT, Le Bert N, Bertoletti A. Difference in sensitivity between SARS-CoV-2–specific T cell assays in patients with underlying conditions. Reply. *J Clin Invest* 2021;131(24):e155701.

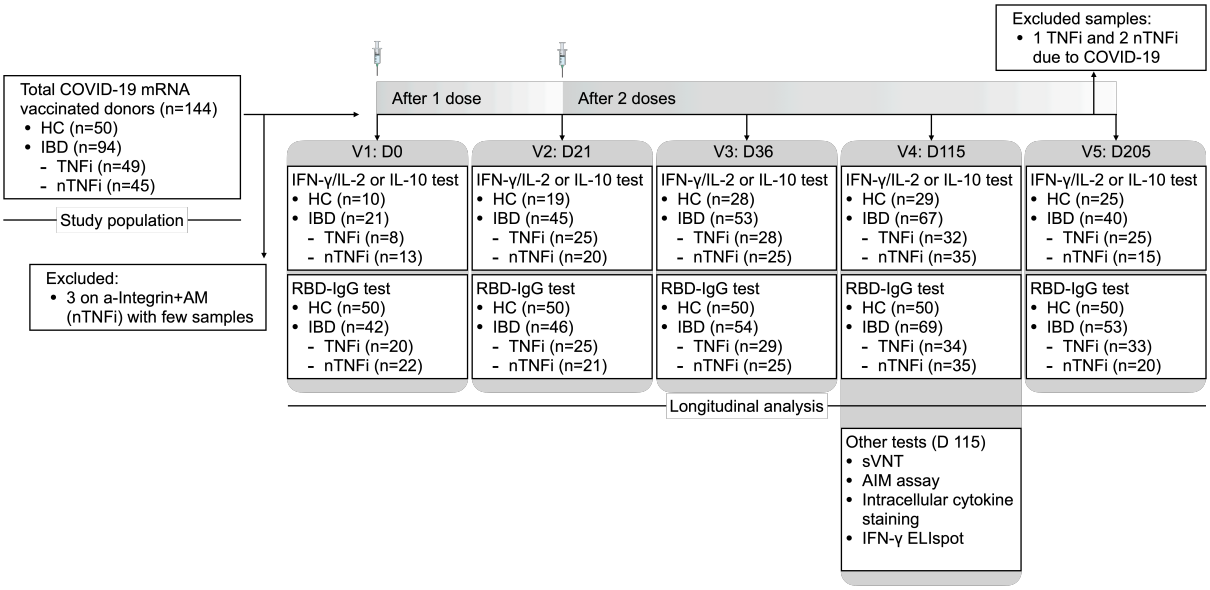


Figure 1. Study design outline. Peripheral blood samples from healthcare workers serving as HCs not on immune-modifying therapy or IBD patients on varying immunotherapies were collected for up to 5 study timepoints of interest. Humoral, cellular, and IL-10 responses were quantified longitudinally. Additional tests including sVNT, AIM assay, intracellular cytokine staining and IFN-γ ELIspot were performed on a subset of donor samples from V4 or D 115 for further analysis.

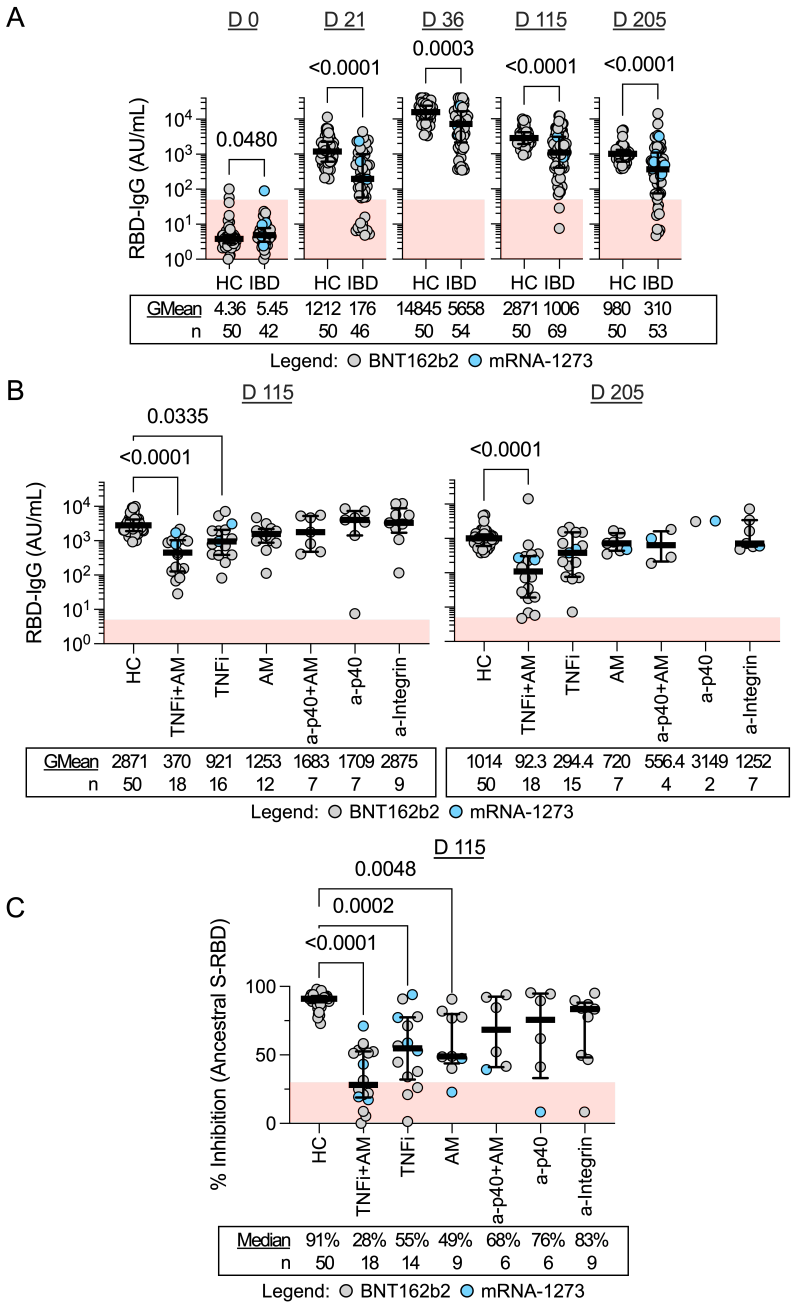


Figure 2. **Humoral immunity is induced following COVID-19 mRNA vaccination.** (**A**-**C**) Dot plots with median line (middle bar) and interquartile range (whiskers) of RBD IgG concentrations (AU/mL) from serum samples of the 2 study cohorts collected at different timepoints (**A**)**,** RBD IgG concentrations (AU/mL) from serum samples of HC and IBD patients grouped by treatment 3 and 6 months after completing their two-dose vaccination (**B**), and % inhibition measured by sVNT of the ancestral SARS-CoV-2 S-RBD from serum samples of HC and IBD patients grouped by treatment 3 months after completing their two-dose vaccination (**C**). Shaded red region denotes the area under the threshold for a positive test. Statistical analyses were performed by Wilcoxon signed rank test (**A**) or by Kruskal-Wallis and Dunn's test (**B** and **C**) with p values indicated above the comparison line when significant (α=0.05). Geometric means (GMean) or Median inhibition and number of data points (n) are indicated below each group.

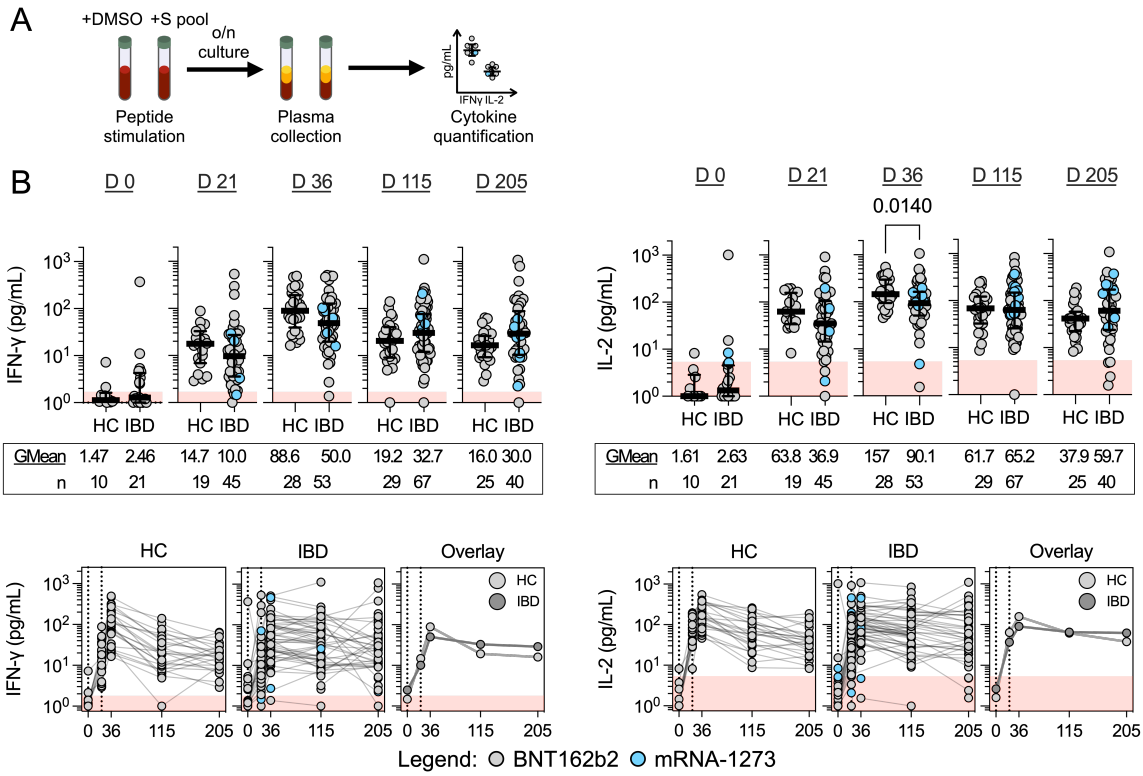


Figure 3. Cellular immunity is induced following COVID-19 mRNA vaccination. (**A**) Overview of whole blood cytokine release assay for IFN-γ/IL-2 quantification. (**B**) Top panel: Dot plots with median line (middle bar) and interquartile range (whiskers) of IFN-γ (left) or IL-2 (right) concentrations (pg/mL) from S pool-stimulated whole blood supernatants of the 2 study cohorts collected at different timepoints. Statistical analyses were performed by Wilcoxon signed rank test with p values indicated above the comparison line when significant (α=0.05). Geometric means (GMean) and number of data points (n) are indicated below each group. Bottom panel: Quantified IFN-γ or IL-2 concentrations (pg/mL) plotted against time, faceted by the two study cohorts. Datapoints originating from the same participant are connected by gray lines. Data is summarized in the 'Overlay' plot with lines connecting the geometric means of each group at each sampling interval. For all graphs, shaded red regions denote the area under the threshold for a positive test.

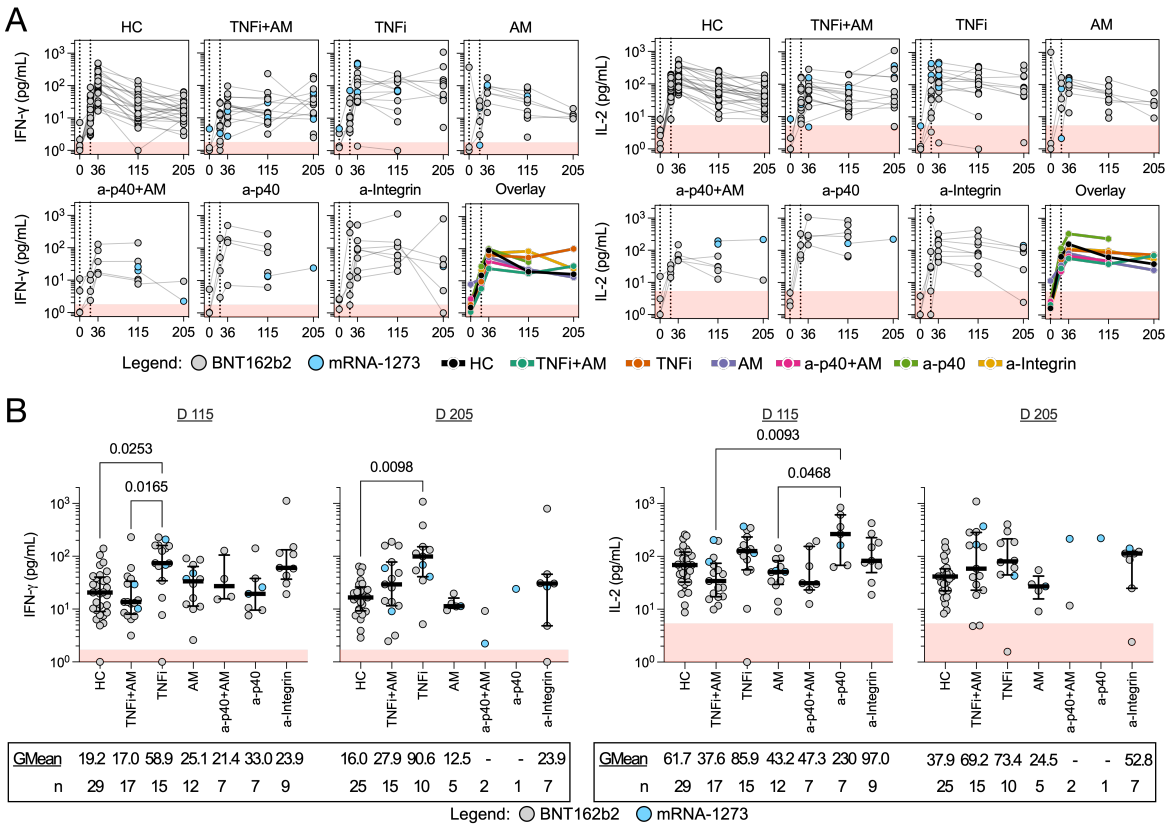


Figure 4. Durable T cell responses are demonstrated by patients under different immunotherapies. (**A**) Quantified IFN-γ or IL-2 concentrations (pg/mL) plotted against time of HC and IBD patients grouped by treatment. Datapoints originating from the same participant are connected by gray lines. Data is summarized in the 'Overlay' plot with lines connecting the geometric means of each group at each sampling interval. (**B**) Dot plots with median line (middle bar) and interquartile range (whiskers) of quantified IFN-γ or IL-2 concentrations (pg/mL) from S pool-stimulated whole blood supernatants of HC and IBD patients grouped by treatment 3 and 6 months after completing their two-dose vaccination. Statistical analyses were performed by Kruskal-Wallis and Dunn's test with p values shown above the comparison lines when significant (α=0.05). Geometric means (GMean) and number of data points (n) are indicated below each group. (**A** and **B**) Shaded red region denotes the area under the threshold for a positive test.

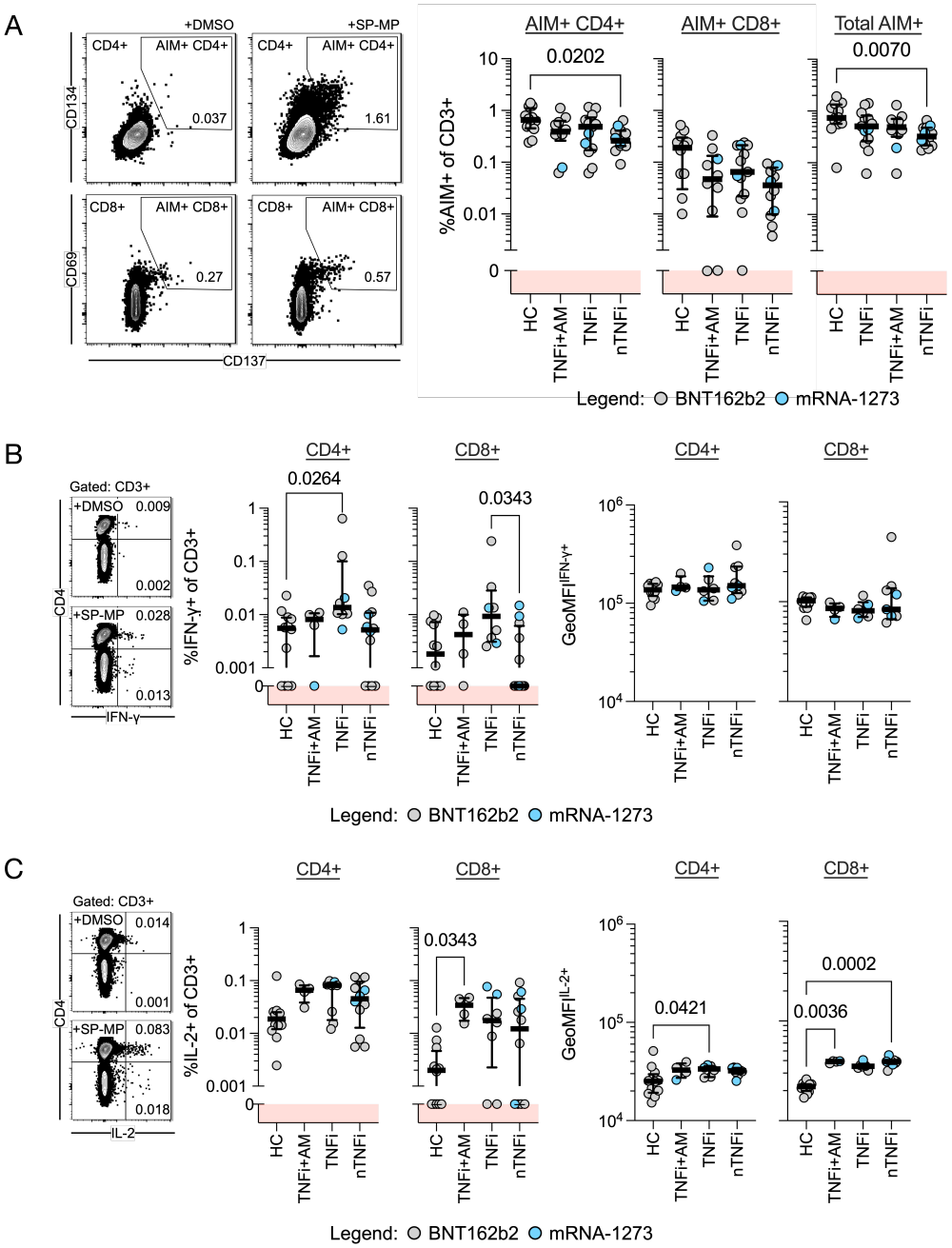


Figure 5. Spike-specific T cells are activated and produce Th1 cytokines. (**A**) Left: Representative flow cytometry plots from AIM assay to identify CD4+CD134+CD137+ and CD8+CD69+CD137+ T cell populations with or without stimulation with Spike peptides. Activated cells are defined by the drawn gate within each population. Right: Summary frequencies of AIM+ cells identified from HC or IBD patient PBMCs 3 months after the second vaccine dose (HC: n=12, TNFi+AM: n=10, TNFi: n=13, nTNFi: n=12). Shaded red regions denote responses below background levels (0). (**B** and **C**) Intracellular cytokine staining for IFN-γ+ (**B**) or IL-2+ (**C**) T cell populations with or without stimulation with Spike peptides. Left: Representative flow cytometry plots. Center/Right: Dot plot (with median line and IQR) summaries of CD4+ or CD8+ IFN-γ+ or IL-2+ frequencies of CD3+ cells and geometric mean fluorescence intensity (GeoMFI) of IFN-γ+ or IL-2+ (with IFN-γ- or IL-2- GeoMFIs subtracted) populations (HC: n=12, TNFi: n=8, TNFi+AM: n=4, nTNFi: n=12). Statistical analyses were performed by Kruskal-Wallis and Dunn's test with p values shown above the comparison lines when significant (α=0.05). Shaded red regions denote responses below background levels (denoted with 0).

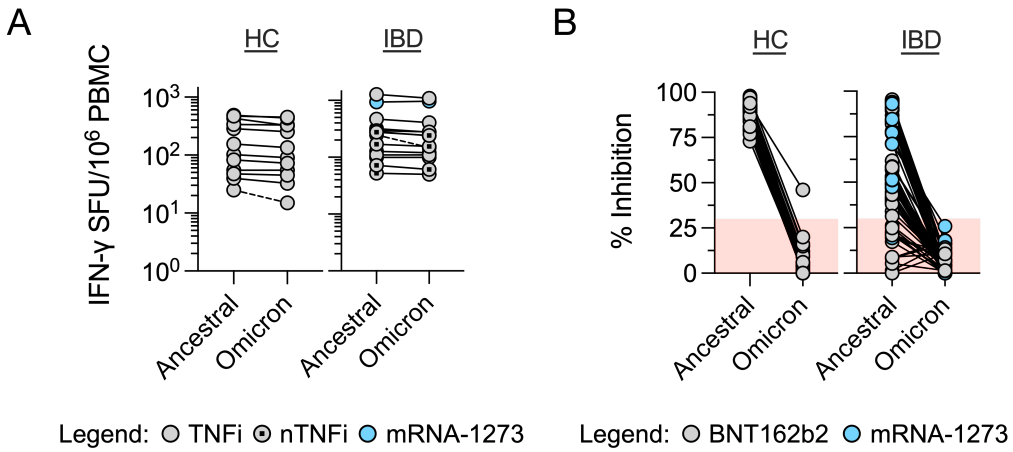


Figure 6. Cellular but not humoral responses are mostly preserved against Omicron variant Spike. (**A**) Dot plots denoting the number of IFN-γ SFU per 10^6 PBMCs generated after ancestral and Omicron variant Spike peptide stimulation in HC or IBD donor groups (D 115). Each line connects paired responses from a single donor, with broken lines denoting a difference of >25% responses. (HC: n=12, TNFi or TNFi+AM (TNFi±AM): n=8, nTNFi: n=6). (**B**) Dot plots denoting the % inhibition of binding of SARS-CoV-2 S-RBD to hACE2 by sVNT from donor sera (D 115). Each line connects paired responses from a single donor (HC: n=50, IBD: n=63).

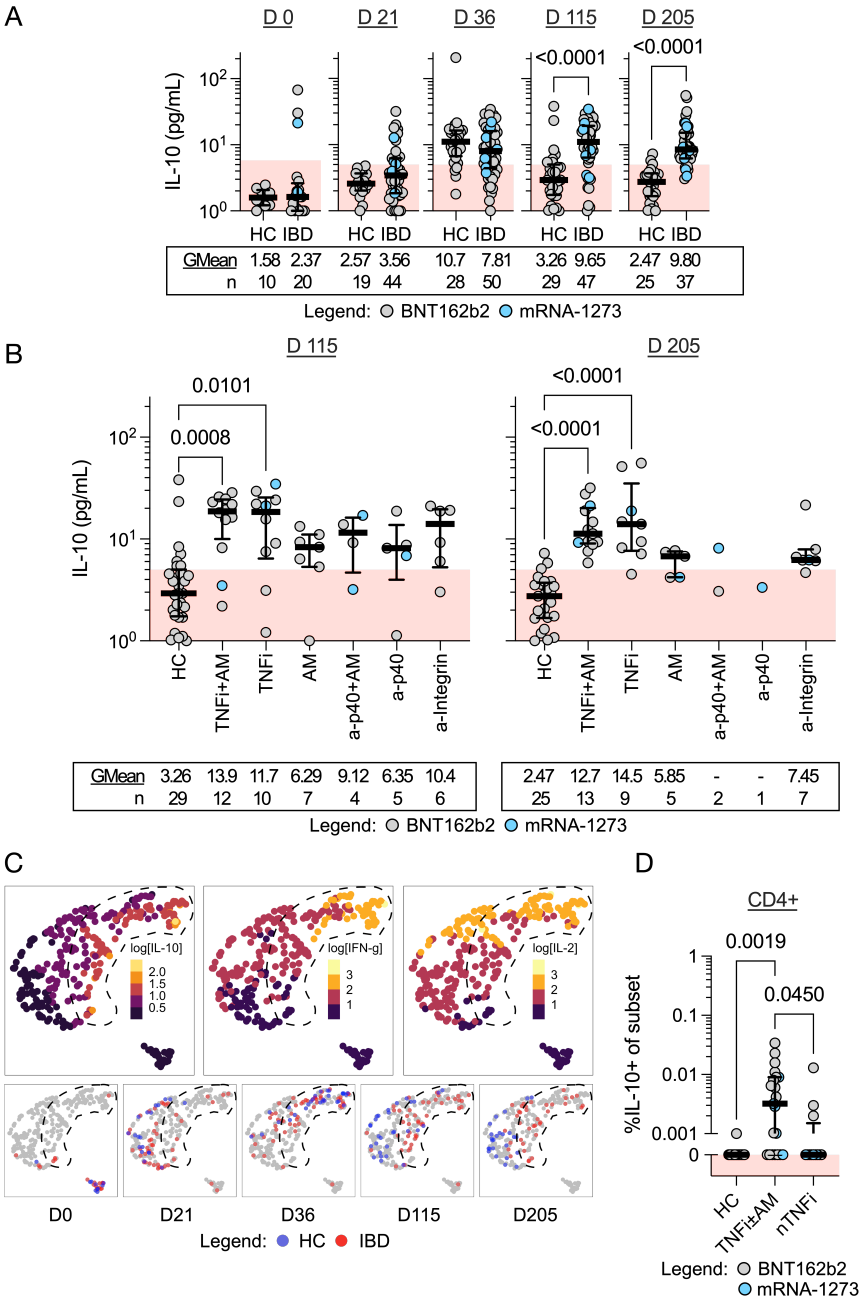


Figure 7. IL-10 delineates T cell cytokine response profile of individuals undergoing immune-modifying therapies. (**A** and **B**) Dot plots with median line (middle bar) and interquartile range (whiskers) of IL-10 concentrations (pg/mL) from S pool-stimulated whole blood supernatants of the 2 study cohorts collected at different timepoints (**A**) and of HC and IBD patients grouped by treatment 3 and 6 months after completing their two-dose vaccination (**B**). Shaded red regions denote the area under the threshold for a positive test. Statistical analyses were performed by Wilcoxon signed rank test (**A**) or by Kruskal-Wallis and Dunn's test (**B**) with p values indicated above the comparison line when significant (α=0.05). Geometric means (GMean) and number of data points (n) are indicated below each group. (**C**) UMAP projections based on IL-10, IFN-γ and IL-2 quantities measured from each donor-timepoint. Top three panels display each point filled according to log10-transformed cytokine quantities (pg/mL). Bottom four panels display points filled according to study cohort at the respective timepoint. A shape is drawn enclosing a region mostly containing points with IL-10 values greater than 10 pg/mL. (**D**) Dot plots with median line (middle bar) and interquartile range (whiskers) of IL-10+ CD4+ cell frequencies of CD4+ from HC (n=12) or IBD donors on TNFi (with or without AM, n=21) or nTNFi therapy (n=12). Statistical analysis was performed by Wilcoxon signed rank test with p values indicated above the comparison line when significant (α=0.05). Shaded red regions denote responses below background levels (denoted with 0).

Table 1. IBD patient demographics grouped according to TNF inhibitor status.

	Total	TNFi	nTNFi	p value
No. of participants (N%)	94	49 (52)	45 (48)	
Age (Median, Range)	41 (21-83)	39 (21-83)	43 (21-79)	0.18[a]
Male (N%)	63	33 (67.3)	30 (66.7)	0.94[b]
Female (N%)	31	16 (32.7)	15 (33.3)	
Race/ethnicity (N%)				
Asian	93	48 (98.0)	45 (100)	
White	1	1 (2.0)	0 (0.00)	
Duration of diagnosis (Median, Range)	12 (2-51)	12 (2-51)	12 (3-37)	0.71[a]
Types of vaccine received (N%)				0.92[b]
BNT162b2	79	41 (83.7)	38 (84.4)	
mRNA-1273	15	8 (16.4)	7 15.6)	
Types of disease (N%)				0.11[b]
Ulcerative colitis	31	12 (24.5)	19 (42.2)	
Crohn's disease	63	37 (75.5)	26 (57.8)	
IBD treatment (N%)				
TNF inhibitor (TNFi)				
Monotherapy		22 (44.9)		
Combination		27 (55.1)		
Other				
Antimetabolite (AM) only			15 (33.3)	
Biologics monotherapy			18 (40.0)	
Anti-p40			7 (15.6)	
Anti-integrin			11 (24.4)	
Biologics combination			12 (26.7)	
Anti-p40+AM			9 (20.0)	
Anti-integrin+AM			3 (6.67)	
Corticosteroid therapy (N%)		2	4	0.60[b]

[a] Wilcoxon signed rank test

[b] Chi-squared test

Table 2. IBD classification by location and behavior grouped according to TNF inhibitor status.

	Total	TNFi	nTNFi	p value
Types of disease (N%)				0.11[a]
Ulcerative colitis	31	12 (24.5)	19 (42.2)	
(Location)				
Extensive disease	15	7 (58.3)	11 (57.9)	
Left sided	9	5 (41.6)	6 (31.6)	
Proctitis/pouchitis	2	0 (0.0)	2 (10.5)	
Crohn's disease	63	37 (75.5)	26 (57.8)	
(Location)				
Ileal	7	5 (13.5)	2 (7.7)	
Ileocolonic	44	24 (64.9)	20 (76.9)	
Colonic	12	8 (21.6)	4 (15.4)	
(Behavior)				
Inflammatory	24	15 (40.5)	9 (34.6)	
Stricturing	21	13 (35.1)	8 (30.8)	
Penetrating	18	9 (8.1)	9 (34.6)	
Perianal disease	15	9 (8.1)	6 (23.1)	

[a] Chi-squared test