Humoral and Cell-Mediated Immunogenicity of COVID-19 Vaccines in Patients with Inflammatory Bowel Disease

By

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ABSTRACT

Patients with inflammatory bowel disease (IBD) may be at increased risk for severe COVID-19. The efficacy of a candidate COVID-19 may be lower in immunosuppressed patients with IBD, since these patient populations may have lower responses to vaccines. Multiple studies have shown that patients with IBD have variability in their vaccine responses compared to healthy individuals. Thus, there is a critical need to determine whether patients with IBD will mount normal immune responses to a COVID-19 vaccine. The overall objective of this proposal is to evaluate the safety and immunogenicity of a COVID 19 vaccine in patients with IBD. This study will help determine whether immunosuppressive regimens impact COVID vaccine response. Furthermore, we will determine whether certain groups may need additional doses of a vaccine, future adjuvanted vaccines, or require a booster to maintain immunity.

We will evaluate these critical questions in the following specific aims:

Aim 1. Determine the immunogenicity and safety of a COVID-19 vaccine in patients with IBD. To achieve this aim we will perform a prospective study evaluating the immunogenicity of a COVID-19 vaccine in 164 patients with IBD. Hypothesis: Certain immunosuppressive regimens such as Anti- TNF in combination with an immunomodulator, will blunt the immunogenicity of a COVID- 19 vaccine, whereas other agents such as immunomodulators or vedolizumab, will not affect the vaccine response.

Aim 2. Determine the impact of immunosuppression on sustained antibody COVID 19 concentrations in patients with IBD who received the COVID 19

vaccine. To achieve this aim we will evaluate sustained antibody concentrations using a quantitative assay from LabCorp that is currently being used by the Centers for Disease Control and Prevention (CDC) to evaluate seroprevalence and study immunity at 1, 6, and 12 months after completion of vaccination. Hypothesis: Vaccine-induced antibody concentrations will wane over the 12 month interval faster than the published rate for healthcare workers.

AKNOWLEDGEMENTS

Completing a PhD is a significant journey encompassing multiple stages and often spanning several years. It demands unwavering dedication, perseverance, and a thirst for knowledge. My own path began with the crucial step of selecting a field and research topic. I was drawn to the exploration of immune responses to vaccines in patients with inflammatory bowel disease (IBD). However, the onset of the COVID-19 pandemic prompted a refinement of my proposal to focus specifically on evaluating the immune response to COVID-19 vaccines in individuals with IBD. This shift led me to write two research grants that provided funding for the Humoral and Cellular Initial and Sustained Immunogenicity in Patients with IBD (HERCULES) study, which now forms the core of my dissertation.

Throughout this journey, I have been fortunate to receive invaluable support and guidance from numerous individuals. I extend my heartfelt gratitude to my esteemed research mentors and collaborators, including Drs. Wald, Hayney, and Farraye, whose insightful advice and expertise have been instrumental in shaping my research endeavors over the years.

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IRB and Protection of Humans Subjects

All the research papers were approved by the Institutional Review Board at University of Wisconsin School of Medicine and Public Health.

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Chapter 1: Specific Aims

Immunosuppressed populations, which include patients with inflammatory bowel disease (IBD) account for approximately 3% of the population in the United States (US).[1] They are commonly treated with immunosuppressive medications that can increase their risk of respiratory infections such as influenza and invasive pneumococcal disease, both of which are vaccine preventable diseases (VPD).[2] Coronavirus disease 2019 (COVID-19) has resulted in a global pandemic with more than 1.5 million infections and more than 280,000 deaths in the US. In response to the pandemic, vaccine development has moved expeditiously with nearly over 200 COVID-19 vaccine candidates currently under development or in clinical trials. In the US four candidate vaccines are in active Phase III clinical trials (two have finished enrollment) and six are in Phase I/II clinical trials. Thus, it is likely that at least one candidate vaccine will become available in the coming weeks under Emergency Use Authorization (EUA) or licensure from the FDA. The efficacy and safety of a candidate vaccine in immunosuppressed populations will not be known since they have been excluded from the current clinical trials, but this population may be included in Phase 1b following healthcare providers for vaccine allocation by the Advisory Committee on Immunization Practices. [3] [4] Thus, there is a *critical need* to evaluate the safety and immunogenicity of a COVID-19 candidate vaccine in patients with IBD.

COVID-19 has a variable spectrum of illness with the majority of infection resulting in asymptomatic or mild disease.[5] However certain healthy adults and immunosuppressed populations can develop severe or critical symptoms that require hospitalization or intensive care stay. Emerging evidence suggest that certain immunosuppressive medications used to treat patients with IBD such as anti-tumor necrosis factor (TNF) do not appear to confer an increased risk for severe COVID-19, but the risk appears to be higher for other agents such as corticosteroids.[6] The efficacy of a candidate COVID-19 may be lower in immunosuppressed patients with IBD, since these patient populations may have lower responses to vaccines.[7-9] Multiple studies have shown that patients with IBD have variability in their vaccine responses compared to healthy individuals.[2,4,9] While many patients have a normal vaccine response, those treated with TNF agents or combination therapy (TNF inhibitors and immunomodulators) are more likely to mount a poor immune response.[10-13] Furthermore, preliminary data suggest some novel regimens (such as ustekinumab, vedolizumab) may not impact the immune response but there is insufficient data to know their impact on vaccine response.[9,14] Thus, there is a *critical need* to determine whether patients with IBD will mount normal immune responses to a COVID 19 vaccine. The overall objective of this proposal is to evaluate the safety and immunogenicity of a COVID 19 vaccine in patients with IBD. This will help determine if immunosuppressive regimens impact COVID vaccine response. Furthermore, we will determine if certain groups may need more doses of a vaccine, with future adjuvanted vaccines or require a booster to maintain immunity. We will evaluate these critical questions in the following specific aims:

Aim 1. Determine the immunogenicity and safety of a COVID-19 vaccine in patients with IBD. To achieve this aim we will perform a prospective study evaluating the immunogenicity of a COVID-19 vaccine in 164 patients with IBD *Hypothesis:* Certain immunosuppressive regimens such as Anti-TNF in combination with an immunomodulator or mycophenolic acid, associated with the lowest vaccine response, will blunt the immunogenicity of a COVID-19 vaccine while other agents such as immunomodulators, tacrolimus, or vedolizumab will not affect the vaccine response.

Aim 2. Determine the impact of immunosuppression on sustained antibody COVID 19

concentrations in patients with IBD who received the COVID 19 vaccine. To achieve this aim we will evaluate sustained antibody concentrations using a quantitative assay from LabCorp that is currently being used by the Centers for Disease Control and Prevention (CDC) to evaluate seroprevalence and study immunity at 1, 6, and 12 months after completion of vaccination. *Hypothesis:* Vaccine-induced antibody concentrations will wane over the 12 month interval faster than the published rate in healthcare workers.

At the completion of the proposed research, we will have determined the safety and immunogenicity of a COVID 19 vaccine in immunosuppressed patients with IBD. Furthermore, we will have determined the effect of different immunosuppressive regimens on sustained immunity to COVID-19 vaccine.

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Chapter 2: Humoral Immunogenicity of mRNA COVID 19 Vaccines Among Patients With Inflammatory Bowel Disease and Healthy Controls

Preface:

This study showed that most patients with inflammatory bowel disease (IBD) are able to mount a humoral immune response (antibody) to the primary series of a COVID-19 vaccine despite being on immunosuppressive therapy. Patients with IBD on tumor necrosis factor (TNF) antagonist had lower antibodies compared to patients on other therapies. Furthermore, patients with IBD had lower antibodies compared to healthy controls.

Citation:

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Title: Humoral Immunogenicity of mRNA COVID 19 Vaccines Among Patients With Inflammatory Bowel Disease and Healthy Controls

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Potential competing interests: Dr. Freddy Caldera has received research support from Takeda Pharmaceuticals and Sanofi. He has been a consultant for Takeda, Arena Pharmaceuticals, GSK, and Celgene. Dr. Farraye is a consultant for Arena, BMS, Braintree Labs, Gilead, GSK, Innovation Pharmaceuticals, Iterative Scopes, Janssen, Pfizer and Sebela. He sits on a DSMB for Lilly and Theravance. Dr. Hayney is a consultant for GSK Vaccines and Seqirus and has received research support from Takeda Pharmaceuticals, Dynavax, and Sanofi. Dr. Chun is an employee of LabCorp.

Keywords: Crohn's disease, Ulcerative Colitis, immune response, antibodies

Abstract

Introduction

Patients with inflammatory bowel disease (IBD) on immune modifying therapies may have a lower vaccine response to certain vaccines. The aim of our study was to evaluate humoral immunogenicity of mRNA COVID-19 vaccines among patients with IBD and healthy controls (HC).

Methods

We performed a prospective study to evaluate humoral immunogenicity among patients with IBD and HC after completion of mRNA COVID-19 vaccines.

Results

One hundred twenty-two patients with IBD and 60 HC were enrolled. All HC and 97% of patients with IBD developed antibodies. Antibody concentrations were lower in patients with IBD compared to HC (median 31 vs. 118 μg/ml; p<0.001). Those who received the mRNA-1273 (Moderna) COVID-19 (median 38; IQR 24-75 vs μg/ml) had higher antibody concentrations compared to those who received the Pfizer-BNT vaccine series (median 22; IQR 11-42 μg/ml; p<0.001). Patients on immune modifying therapy (median 26; IQR 13-50 μg/ml) had lower antibody concentrations compared to those who were on no treatment, aminosalicylates or vedolizumab (median 59; IQR 31-75 μg/ml; p=0.003).

Discussion

Almost all patients with IBD in our study mounted an antibody response. Future studies are needed evaluating sustained humoral immunity and the impact of booster dosing in patients with IBD.

Introduction

Three highly effective coronavirus disease 2019 (COVID-19) vaccines are currently available under an FDA emergency use authorization (EUA) for all adults to help reduce the morbidity and mortality from COVID-19 disease.(1) Whether these vaccines are equally effective for patients with inflammatory bowel disease (IBD), who are commonly on immune modifying therapy which may blunt the immune response of certain vaccines, has been a concern since the vaccines were made available.(2) Studies of solid organ transplant recipients have shown suboptimal humoral immune responses after immunization with either the BNT162b2 (Pfizer-BioNTech) or mRNA-1273 (Moderna) COVID-19 (mRNA) vaccine series. In a study of 658 transplant recipients, only 54% of patients developed anti-spike antibodies evaluated via a commercial assay.(3)

In contrast, preliminary results among patients with IBD suggest that the majority of patients with IBD produce antibodies after completing the mRNA COVID-19 vaccine series. Initial analysis of the PREVENT-COVID and CORALE-IBD study showed that 95% and 100% of patients with IBD had a humoral antibody response. (4, 5) Despite the reassurance in these studies that patients with IBD respond to the vaccine by generating anti-spike antibodies, control groups were not included making it difficult to determine if responses were generally normal or suboptimal. Thus, we assessed humoral immunogenicity among patients with IBD and healthy controls after COVID-19 vaccination at time points similar to the initial COVID-19 vaccine trials. (6)

Materials and Methods

This non-blinded multicenter study (HERCULES) evaluated the humoral immunogenicity of mRNA COVID-19 vaccines among patients with IBD seen at University of Wisconsin-Madison and healthy controls (HC) from LabCorp. Eligibility criteria for patients with IBD were a diagnosis of IBD, ages 18-85 years, on stable doses of maintenance therapy at least two months (see supplementary methods), and completion of an mRNA vaccines series confirmed using the Wisconsin Immunization Registry. HC were eligible if they were not on immunosuppressive therapy and had documentation that they completed an mRNA vaccine series. All participants had no clinical history of COVID-19 infection, and those with serological evidence of asymptomatic infection were not eligible.

Nucleocapsid and spike protein S1 receptor binding domain (RDB)-specific IgG antibodies were measured in sera at 28-35 days post completion of the two dose mRNA series in patients with IBD and at approximately 30 days in HC similarly to COVID-19 immunogenicity clinical trials. (Supplementary methods for full details). (6)

The study received IRB approval at University of Wisconsin and LabCorp.

Results

One hundred twenty-two patients with IBD and 60 HC were enrolled. Characteristics of the groups were similar, except for the distribution of the mRNA vaccine preparations. (Table 1)

The HC group had higher antibody concentrations (median 118 (interquartile range (IQR) 87-189 μg/ml) at one month after completing the mRNA vaccine series compared to the patients with IBD (median 31 (IQR 16-63) μg/ml; $p<0.001$). (Figure 1) However, only 4 of 122 (3%) patients

with IBD, all of whom were on immunosuppressing therapies, failed to mount a measurable antibody response. All healthy controls had an antibody response. Among the patients with IBD, those who received the Moderna COVID-19 mRNA vaccine series (median 38; IQR 24-75 vs μg/ml) had higher antibody concentrations compared to those who received the Pfizer-BNT mRNA vaccine series (median 22; IQR 11-42 μg/ml; $p<0.001$). Patients who were immunosuppressed (defined as treated with thiopurines, anti-TNF agent, ustekinumab, tofacitinib, or corticosteroids) (median 26; IQR 13-50 μg/ml) had lower antibody concentrations compared to those who were on no treatment, aminosalicylates or vedolizumab (median 59; IQR 31-75 μg/ml; $p=0.003$). (Supplementary Figure 1)

Discussion

In our study almost all patients with IBD developed a humoral immune response after completing their mRNA vaccine series similar to HC. This, along with other studies, supports the finding that patients with IBD despite being on immune modifying therapy still mount a humoral immune response and are able to achieve seropositivity after completing a two dose mRNA series. However, we found that patients with IBD had lower antibody concentrations compared to HC at approximately one month after the second vaccination. Lower neutralizing antibody concentrations were associated with asymptomatic or mild breakthrough infections with SARS-CoV2 in thirty fully vaccinated health care workers. (7)

Not surprisingly, patients with IBD who are treated with immunosuppressing medications have lower vaccine-induced antibody concentrations than HC. (8) However, a lower antibody concentration does not necessarily mean lower protection or lack of immunity since a correlate

of protection for COVID-19 vaccines has yet to be determined. Thus, until a correlate of protection for COVID-19 vaccines is identified, the Advisory Committee on Immunization Practice will continue to use the best evidence available to advise clinicians on additional COVID-19 doses and boosters.

The FDA recently expanded the EUA to allow patients who have the equivalent immunosuppression as solid organ transplant recipients to receive an additional dose of an mRNA vaccine. Accumulated data on patients with IBD thus far indicate that this group of immunosuppressed individuals do not require an additional mRNA dose to achieve seropositivity at one month since 97% of our patients and (95-100%) of patients in PREVENT-IBD and CORALE-IBD achieved seropositivity. We were not able to compare our antibody concentrations to other IBD or HC studies, because antibody concentrations were measured with different assays and collected at different time points following immunization across the studies. We found that patients with IBD who received the mRNA-1273 (Moderna) COVID-19 series had higher antibody concentrations compared to those who received BNT162b2 (Pfizer-BioNTech) series. The importance of this finding needs to be evaluated in larger IBD cohorts and other immunosuppressed populations since the mRNA-1273 was found to have higher vaccine effectiveness against COVID-19 hospitalization and induce higher post vaccination anti-RDB antibody levels than BNT162b2 in healthy volunteers in a recent report from the Centers for Disease Control and Prevention. (9)

The FDA and ACIP now recommends a booster with BNT162b2 for all adults 65 years and older, and adults 50-64 years with underlying medical conditions. In addition, clinicians may offer a booster dose to 18-48 year olds with underlying medical conditions or to adults 18-64

who are at increased risk for COVID-19 exposure and transmission because of occupational or institutional setting. (10) This booster should be given at least six months after completing the initial vaccine series. It is anticipated that boosters for m-RNA-1273 will also be recommended in the future. Whether patients with IBD, especially those on immunosuppressive medications, will need more than three doses of an mRNA series to prevent severe disease and/or breakthrough infection is not known since the majority of studies have only evaluated antibody concentrations up to two months following completion of the primary series. However, because their vaccine-induced antibody concentrations are lower compared to healthy individuals, investigations into the need for and timing of additional booster doses should be a high priority. A recent report showed that antibodies wane after the second dose of BNT162b2 in HC.(11) Thus, it will important to determine if antibodies wane faster in individual on immune modifying therapy especially if it is determined that neutralizing antibodies are deemed predictive of immune protection against severe disease or break through infections. (12)

Our study has several strengths. We evaluated humoral immunogenicity at one month following the COVID-19 vaccine series completion, similar to the immunogenicity clinical trials, in patients on stable treatment regimens and compared our results to healthy controls. (13) Our study is limited by its sample size and small representation of certain treatment regimens.

In conclusion, the vast majority of patients with IBD achieved seropositivity after completing the mRNA vaccine series. Further studies are needed evaluating initial cell-mediated immunogenicity and long-term studies evaluating sustained antibody concentrations, cell

mediated immunity, and incidence of breakthrough infections to determine the need for and timing of booster doses in patients with IBD.

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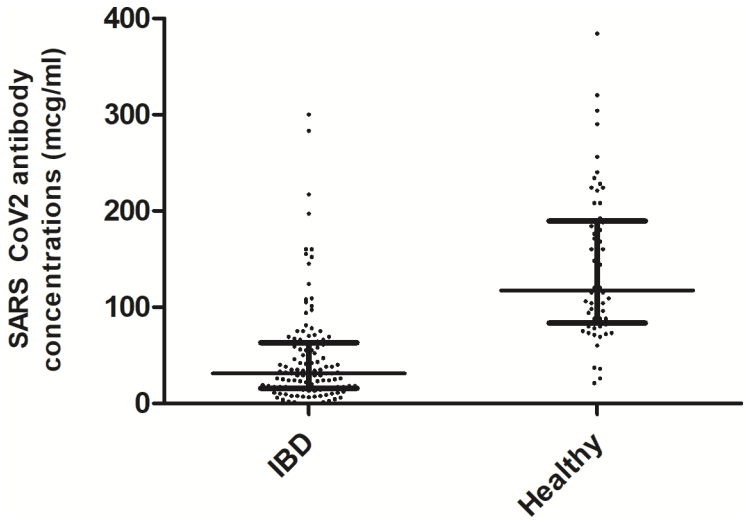

Figure 1 legend

SARS CoV antibody concentrations among inflammatory bowel disease (IBD) treatment groups. SARS CoV2 antibody concentrations higher in healthy controls (median 118 (interquartile range (IQR) 87-189 µg/ml) compared to all patients with IBD (median 31 (IQR 16-63) µg/ml; $p<0.001$) Scatterplot lines show median and 25th and 75th percentile.

Table 1. Study participant characteristics

	IBD Patients N=122	Healthy Controls N=60	P value
Age in years (median (IQR*))	40 (33-52)	38 (30-49)	0.30
Male (%)	64 (52%)	26 (43%)	0.25
Vaccine Manufacturer			
Moderna	62(51%)	52 (86%)	<0.001
Pfizer	59 (48%)	7 (12%)	
Missing		1 (2%)	
Type of IBD			
Crohn's Disease (%)	85 (70%)	--	
Ulcerative Colitis (%)	37 (30%)	--	
IBD treatment*			
No IBD therapy	8 (7%)		
Mesalamine monotherapy	10 (8%)	--	
Vedolizumab monotherapy	10 (8%)	--	
Thiopurine	6 (5%)	--	
antiTNF therapy	46 (38%)	--	
antiTNF combination	19 (16%)	--	
Ustekinumab monotherapy or combination	11 (9%)	--	
Tofacitinib	6 (5%)	--	
Corticosteroid therapy (2.5-40mg/day)	5 (4%)	--	
Duration of immunosuppression n=95 (mean ± SD)	69 ± 76		
Antibody concentrations (mg/ml; mean + SD)	31 (16-63)	118 (87-189)	<0.001
Antibody response	118 (97%)	60 (100%)	0.30
Vaccine type			
Pfizer; n=60	22 (11-42)		<0.001
Moderna; n=62	38 (24-75)		
Biologic			
Yes; n =90	26 (14-49)		0.009
No; n=32	57 (21-75)		
Immunosuppressed **			
Yes; n =95	26 (13-50)		0.003

No; n=27	59 (31-75)		

*Sums to more than 100% as all 5 patients on corticosteroids are also on other medications (3 on antiTNF and 2 on ustekinumab)
**Immunosuppressed includes all patients treated with thiopurines, antiTNF, ustekinumab, tofacitinib or corticosteroids.
Abbreviations: IBD: inflammatory bowel disease, IQR: interquartile range, antiTNF: tumor necrosis factor blocking agent

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Chapter 3: Humoral Immunogenicity of Three COVID-19 mRNA Vaccine Doses in Patients with Inflammatory Bowel Disease

Preface:

We evaluated the humoral immunogenicity of a third COVID-19 mRNA vaccine dose in patients with inflammatory bowel disease. All patients displayed a humoral immune response and median antibody concentrations were higher after the third dose than after completion of the two-dose series.

Citation:

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Title:

Humoral Immunogenicity of Three COVID-19 mRNA Vaccine Doses in Patients with Inflammatory Bowel Disease

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Specific author contributions:

T.L.S. – acquisition of data, analysis and interpretation of data, drafting of the manuscript, and critical revision of the manuscript. F.C. – study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, and critical revision of manuscript. K.L.K – analysis and interpretation of data and critical revision of manuscript. S.S. – critical revision of manuscript. A.W. – critical revision of

manuscript. H.S.P – acquisition of data, critical revision of manuscript. M.A. – acquisition of data, critical revision of manuscript. K.C. – acquisition of data, critical revision of manuscript, I.G. – critical revision of manuscript. M.L. – critical revision of manuscript. M.S.H – study concept and design, acquisition of data, analysis, and interpretation of data, drafting of the manuscript, and critical revision of manuscript. F.A.F. – analysis and interpretation of data and critical revision of manuscript.

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Dr. Freddy Caldera has received research support from Takeda Pharmaceuticals. He has been a consultant for Takeda, Arena Pharmaceuticals, GSK, and Celgene. Dr. Farraye is a consultant for Arena, BMS, Braintree Labs, GSK, Innovation Pharmaceuticals, Iterative Scopes, Janssen, Pfizer and Sebela. He sits on a DSMB for Lilly and Theravance. Dr. Hayney is a consultant for GSK Vaccines and Seqirus and has received research support from Takeda Pharmaceuticals, Dynavax, and Sanofi. Dr. Chun is an employee of LabCorp.

Keywords:

Inflammatory bowel disease: Crohn's disease: Ulcerative colitis: COVID-19 vaccine

Introduction:

Three safe and effective COVID-19 vaccines were authorized by the Food and Drug Administration in response to the COVID-19 pandemic. Neither patients with inflammatory bowel disease (IBD) nor immunosuppressed patients were included in

the original Phase III clinical trials. Studies have since demonstrated a humoral immune response rate of 95–99% following vaccination with a two-dose mRNA COVID-19 vaccine series in patients with IBD.[1–3] Results show that those on certain immune-modifying therapies, such as anti-tumor necrosis factor (anti-TNF) therapy in combination with an immunomodulator or the use of systemic corticosteroids, may exhibit a relatively diminished humoral immune response and a relative decrease in serum antibody concentrations over time.[1–3]

The Advisory Committee on Immunization Practice (ACIP) recommends a third COVID-19 mRNA vaccine as part of the primary series for those who are moderately-to-severely immunocompromised, or as a booster dose for those who are otherwise immunocompetent.[4] The aim of this study was to evaluate the humoral immunogenicity of a third COVID-19 mRNA vaccine dose in patients with IBD. We hypothesized that patients would mount a significant humoral immune response, and that those on certain immune-modifying therapy such as systemic corticosteroids or anti-TNF combination therapy would have relatively lower serum antibody concentrations.

Methods:

This was a multicenter, prospective, non-randomized study comprised of patients with IBD and healthy controls (HC) in the "HumoRal and CellULar initial and Sustained immunogenicity in patients with IBD" (HERCULES) cohort.[2] Participants with IBD were

enrolled at the University of Wisconsin-Madison (Madison, Wisconsin) and Mayo Clinic (Jacksonville, Florida), while HC were employees of LabCorp. Patient eligibility criteria included a diagnosis of IBD, age 18–85 years, stable doses of maintenance therapy (any IBD-directed therapy used for ≥ 2 months following the induction phase of therapy) or absence of IBD-directed therapy (for ≥ 6 months), and completion of a two-dose mRNA vaccine series. HC eligibility criteria included absence of immunosuppressive therapy and documented completion of a two-dose mRNA vaccine series. A third COVID-19 mRNA vaccine dose was available to patients with IBD but not HC. No participants had clinical history of COVID-19 infection, and those with laboratory evidence of prior infection, as demonstrated by presence of SARS-CoV-2 nucleocapsid antibodies, were excluded.

Participants recruited at Mayo Clinic received their COVID-19 vaccines at a Mayo Clinic facility, and receipt of vaccine was confirmed by interview and review of the electronic medical record. At the University of Wisconsin, vaccination status was confirmed by review of the Wisconsin Immunization Registry (WIR). The WIR is a state-wide database maintained by the Department of Health and Family Services of the State of Wisconsin in which vaccine data for each Wisconsin resident is stored. The WIR captures 97% of vaccines administered in the state, and 98.5% of Wisconsin residents have an active WIR record. The WIR does not capture vaccines administered outside the state, and all Wisconsin vaccine providers are required to enter record of COVID-19 vaccine administration into the registry.[5] The WIR has been previously been used to evaluate COVID-19 vaccine uptake in patients with IBD.[6]

The primary outcome was total serum SARS-CoV-2 anti-spike IgG antibody concentrations following a third dose compared to antibody concentrations following the two-dose series in the IBD cohort. Secondary outcomes included antibody concentrations following a third dose in patients with IBD compared to antibody concentrations 180 days after the two-dose series in HC. The effects of vaccine manufacturer and immunosuppressive therapy on antibody concentrations following a third dose in patients with IBD were also evaluated.

Specific antibodies measured in sera were nucleocapsid and spike protein S1 receptor-binding domain (RDB)-specific IgG antibodies reported as mcg/ml, as previously described.[2] In patients with IBD, we measured antibody concentrations 28–35 days (t_1) after completion of the two-dose series and 28–65 days (t_2) after the third dose. Only patients with IBD who received a third dose had antibody concentrations measured at t_2, and not every subject who had antibody concentrations measured at t_2 had them measured at t_1 due to timing of enrollment. In HC, we measured antibody concentrations 30 days (t_1) and 180 days (t_2) after completion of the two-dose series. Enzyme-linked immunoassay (ELISA) was performed at Labcorp as previously described.[2]

IBD treatment groups were defined as subjects on stable doses of maintenance therapy as previously described.[2] Non-immunosuppressive therapy was defined as absence of IBD-directed therapy or receipt of treatment with mesalamine monotherapy

or vedolizumab monotherapy. Immunosuppressive therapy was defined as thiopurine monotherapy (i.e., azathioprine, 6-mercaptopurine), anti-TNF monotherapy, anti-TNF combination therapy (i.e., plus antimetabolite), ustekinumab monotherapy or combination therapy, tofacitinib, or systemic corticosteroid therapy (i.e., any of the aforementioned groups plus systemic corticosteroids). Antibody concentrations between groups were compared using Mann-Whitney U tests. The study received IRB approval at the University of Wisconsin, Mayo Clinic, and LabCorp.

Results:

One hundred thirty-nine patients with IBD completed the two-dose series and had antibody concentrations measured at t_1, and 85 patients received a third dose and had antibody concentrations measured at t_2 (Table 1). The median time between receipt of third dose and completion of the two-dose series was 149 days (IQR 132–167). Forty-six HC completed the two-dose series and had antibody concentrations measured at both timepoints. In the IBD cohort, 48.2% and 51.8% received the two-dose Moderna and Pfizer series, respectively, compared to 93.5% and 6.5% in HC ($p<0.001$). Two patients with IBD switched from the Moderna two-dose series to the Pfizer third dose. The median age of patients with IBD was significantly lower than that of HC (median 38 (IQR 30–49) vs 42 (IQR 35–58), $p=0.033$). The age of patients with IBD who received a third dose was significantly greater than that of those who completed only the two-dose series (median 48 (IQR 38–60) vs 41 (IQR 34–52), $p=0.003$). The characteristics of the groups were otherwise similar.

In patients with IBD, antibody concentrations were significantly higher following a third dose in comparison to the two-dose series (median 68 (IQR 32–147) vs 31 (IQR 16–61), p<0.001) (Figure 1A). One hundred thirty-five patients with IBD (97.1%) had detectable antibody concentrations at t_1, while all 85 patients (100%) had detectable antibody concentrations at t_2 (p=0.12). Of the two patients with IBD who were seronegative at t_1 and received a third dose, each had detectable antibody concentrations (mean 6.25, SD 2.1) at t_2; one patient was on anti-TNF monotherapy and the other was on tofacitinib.

At t_2, antibody concentrations were similar between patients with IBD on immunosuppressive therapy and non-immunosuppressive therapy (median 69 (IQR 46–159) vs 66 (IQR 28–147), p=0.27) (Figure 1B). Subgroup analysis revealed that those on systemic corticosteroids, anti-TNF monotherapy, and anti-TNF combination therapy had significantly lower antibody concentrations at t_2 than patients that were not (39 (IQR 20–120) vs 73 (IQR 60–167), p<0.001). Serum antibodies were significantly higher at t_2 for patients with IBD who received three Moderna doses compared to those who received three Pfizer doses (median 94 (IQR 38–170) vs 62 (IQR 31–96), p=0.047).

Although HC had higher antibody concentrations compared to IBD subjects at t_1 (median 120 (IQR 88–190) vs 31 (IQR 16–61), p<0.001), HC had lower antibody concentrations than IBD subjects at t_2 (median 17 (IQR 11–22) vs 68 (IQR 32–147),

p<0.001).

Discussion:

All patients with IBD who received a third COVID-19 mRNA vaccine dose demonstrated a humoral immune response. Median antibody concentrations were higher following a third dose than after the two-dose series. These findings are similar to those reported in other immunosuppressed patient populations with autoimmune disease, but are distinct from solid organ transplant since all patients had an immune response following three COVID-19 mRNA vaccine doses, compared to a humoral immune response rate of 49–68% in solid organ transplant recipients.[7–10] Furthermore, those who completed a three-dose Moderna series had higher antibody concentrations than those who completed a three-dose Pfizer series, which is similar to previously reported findings regarding the two-dose series.[2]

Subgroup analysis revealed that patients on systemic corticosteroids, anti-TNF monotherapy, and anti-TNF combination therapy had relatively lower antibody concentrations than patients who were not, suggesting that immune-modifying therapy may impact the humoral immune response to COVID-19 vaccines as seen in CORALE-IBD, HERCULES, and PREVENT-COVID.[1–3] Our findings that those on anti-TNF monotherapy had lower antibody concentrations is limited due to small sample size as this relationship has not been established in other cohorts. Moreover, this effect may be related to how subgroup analysis was performed being that those on anti-TNF

monotherapy were grouped with those on anti-TNF combination therapy and corticosteroids, medication categories that have been associated with an attenuated humoral response in other vaccines, thus, these subgroup analyses are hypothesis-generating.[11]

It is important to note that difference in antibody concentrations may not be clinically relevant since a correlate of immunity is not known. Measuring serum antibody concentrations evaluates solely the humoral immune response to vaccination, neglecting cell-mediated immunity and other components of a vaccine-induced immune response. Having an antibody response is likely the most relevant serologic endpoint in evaluating humoral immunity since it provides objective evidence of a vaccine-induced immune response. Clinical endpoints are also relevant targets of vaccine efficacy. Receipt of a third COVID-19 mRNA vaccine has increased vaccine effectiveness against hospitalization from 69% to 88% in adults with immunocompromising conditions, including a wide spectrum of conditions.[4]

In August 2021, the ACIP recommended an additional dose to the primary series for those who are moderately-to-severely immunocompromised.[12] This recommendation was largely based on evidence that many solid organ transplant recipients did not mount an antibody response to the primary two-dose series but went on to demonstrate an improved humoral response to a third dose, and these findings were subsequently extrapolated to other similarly immunosuppressed populations.[9,13] This additional dose to the primary series, a three-dose series, is intended for people who

likely did not mount an immune response after initial vaccination. In November 2021, the ACIP recommended a booster dose six months after completion of the two-dose mRNA vaccine series for all adults, and a potential fourth dose for those who are moderately-to-severely immunosuppressed and completed a three-dose series.[12] Supporting evidence for booster doses included evidence of waning humoral immunity in the general population following COVID-19 vaccination (which we observed in our HC cohort),[14] a high incidence of breakthrough infection among vaccinated healthcare workers and the general population,[15,16] and a reduction in the incidence of infection and severity of illness following booster receipt.[17,18] In our cohort, patients received a third dose a median of 149 days after the second dose. Previous studies demonstrated that a two-dose primary series is sufficient to render a humoral immune response in patients with IBD.[2] As such, this study essentially evaluates the humoral effects of booster doses in patients with IBD.

The ACIP recently revised their guidance for booster doses, preferentially recommending an mRNA booster dose 5 months after the primary series for all adults and three months after the third dose for those who are moderately-to-severely immunocompromised.[19] Patients with IBD on thiopurines, anti-TNF therapy, or systemic corticosteroids qualify for a fourth dose per ACIP recommendations.[19] Similar to prior vaccine recommendations from the ACIP for those that are considered moderately-to-severely immunocompromised, much of the data came from the solid organ transplant population with concerns of waning or unmeasurable serum antibodies after three doses of an mRNA vaccine and improved immunogenicity after

a fourth dose.[20] In contrast, it appears that patients with IBD sustain humoral immunity over time, with one study showing that all 75 participants, all of whom were on a form of immune-modifying therapy, maintained measurable serum antibody concentrations six months after a two-dose primary series.[21] It is important to note that patients with IBD have higher rates of humoral immune responses to COVID-19 vaccines than solid organ transplant recipients or those treated with B cell-depleting therapies as described above. Previous studies have described a 95–99% humoral immune response rate following vaccination with a two-dose mRNA vaccine series in patients with IBD.[1–3] Herein, we observed a humoral immune response in all patients following receipt of three doses. Moreover, a recent report from the SECURE-IBD registry demonstrated that among patients with IBD on various forms for IBD-directed therapy, only those on systemic corticosteroids therapy appear to be at greater risk of adverse COVID-19 outcomes.[22] Those who would most likely benefit from a fourth mRNA COVID-19 vaccine dose include solid organ transplant recipients, those on systemic corticosteroids at the time of vaccination, those on concomitant therapies associated with lower COVID-19 vaccines response (mycophenolate or B cell-depleting therapies), those who have comorbidities associated with adverse COVID-19 outcomes, and potentially those who received their third dose roughly ≥ 6 months prior.

Our study has several strengths. We evaluated humoral immunogenicity in patients on stable medication regimens (median 39 months in those who received a third dose), which permitted us to assess the effect of medications on the immune response to vaccination. We also included a HC reference population that received the two-dose

primary series but did not receive a third dose. Our study is limited in its sample size, small representation of certain treatment regimens, and the absence of a reference HC population that received a third dose. Population differences between HC and the IBD cohort in addition to differences between IBD patients that received a third dose and those that did not may have contributed to findings.

In conclusion, all patients with IBD exhibited a humoral immune response following a third COVID-19 mRNA vaccine dose, and this response may be blunted by certain immune-modifying therapy. The role of serum antibody concentrations as a correlate of immunity has not been definitively established. Further studies are needed to investigate the durability of humoral immunity in addition to other aspects of the adaptive immune response following COVID-19 vaccination in the IBD population.

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Table 1: Study participant characteristics			
	IBD subjects Third dose (n=85)[1]	IBD subjects Two-dose series (n=139)[2]	p-value
Demographics			
Age [years] – median (IQR)	48 (38–60)	41 (34-52)	0.003
Male – no. (%)	38 (44.7)	71 (51)	0.35

Type of IBD			
Crohn's Disease – no. (%)	55 (64.7)	96 (69.1)	0.44
Ulcerative Colitis – no. (%)	30 (35.3)	42 (32.2)	
IBD Unclassified	-	1 (0.7)	
Vaccine data			
Two-dose series			
Moderna – no. (%)	37 (43.5)	67 (48.2)	0.58
Pfizer – no. (%)	48 (56.5)	72 (51.8)	
Third dose			
Moderna – no. (%)	35 (41.2)	-	
Pfizer – no. (%)	50 (58.8)	-	
Time between completion of two-dose series and third dose [days] – median (IQR)	149 (132–167)	-	
IBD treatment			
Non-systemic immunosuppression[a] – no. (%)	24 (28.2)	31 (22.3)	0.34
Aminosalicylate or no IBD therapy – no. (%)	3 (3.5)	17 (12.2)	
Vedolizumab monotherapy – no. (%)	21 (24.7)	14 (10.1)	
Duration of therapy [months] – median (IQR)[b]	36 (17-54)	26 (16-47)	0.41
Systemic immunosuppression[c] – no. (%)	61 (71.8)	108 (77.7)	0.34
Thiopurine monotherapy – no. (%)	6 (7.1)	14 (10.1)	
Anti-TNF monotherapy – no. (%)	31 (36.5)	59 (42.4)	
Anti-TNF combination therapy – no. (%)	12 (14.1)	12 (8.6)	
Ustekinumab monotherapy or combination therapy – no. (%)	9 (10.6)	15 (10.8)	
Tofacitinib monotherapy – no. (%)	2 (2.4)	6 (4.3)	
Systemic corticosteroid therapy – no. (%)	1 (1.2)	8 (5.8)	
Duration of therapy [months] – median (IQR)	42 (18-117)	43 (14-85)	0.76
Serum antibody concentrations			
Detectable antibody concentrations – no. (%)	85 (100)	135 (97.1)	0.12

Serum antibody concentrations [mg/mL] – median (IQR)	68 (32–147)	31 (16–6.1)	<0.001
Time between vaccine dose and antibody measurement [days] – median (IQR)	37 (32–47)	32 (29–34)	<0.001

[a] Absence of IBD therapy, mesalamine monotherapy, or vedolizumab monotherapy

[b] Subjects with absence of IBD therapy were omitted from the calculation of duration of therapy

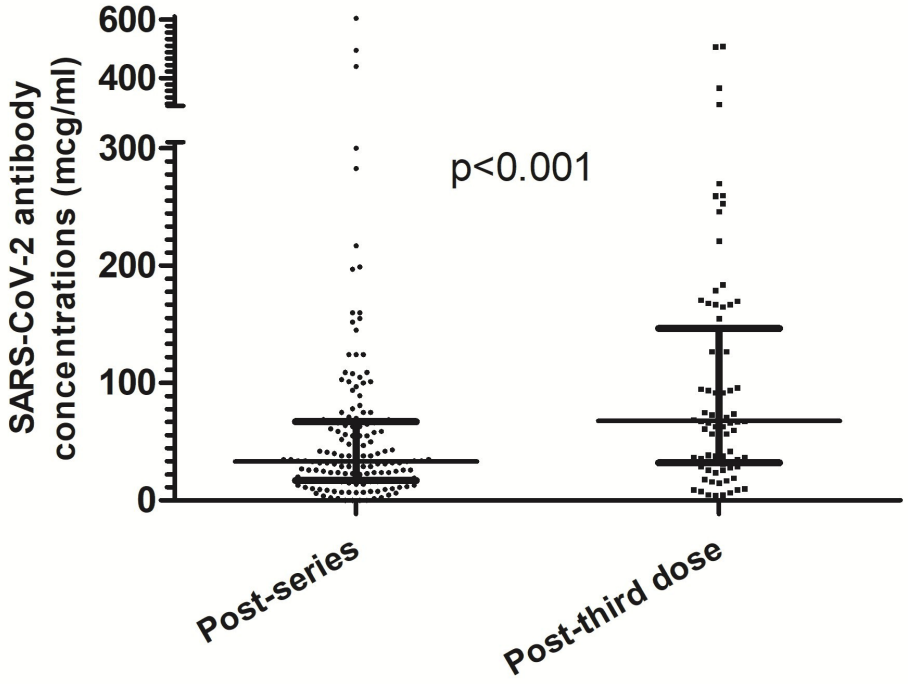
[c] Thiopurine monotherapy (i.e., azathioprine, mercaptopurine), anti-TNF monotherapy, anti-TNF combination therapy (i.e., plus antimetabolite), ustekinumab monotherapy or combination therapy, tofacitinib, or systemic corticosteroid therapy (i.e., any of the aforementioned groups plus systemic corticosteroid)

[1] Completed two-dose series and had serum antibody concentrations measured 28–35 days thereafter

[2] Completed third dose and had serum antibody concentrations measured 28–65 days thereafter

Abbreviations: Inflammatory Bowel Disease (IBD), no. (number), interquartile range (IQR), tumor necrosis factor (TNF)

A


SARS-CoV-2 antibody concentrations (mcg/ml)
600
400
300
200
100
0
p<0.001
Post-series
Post-third dose

B

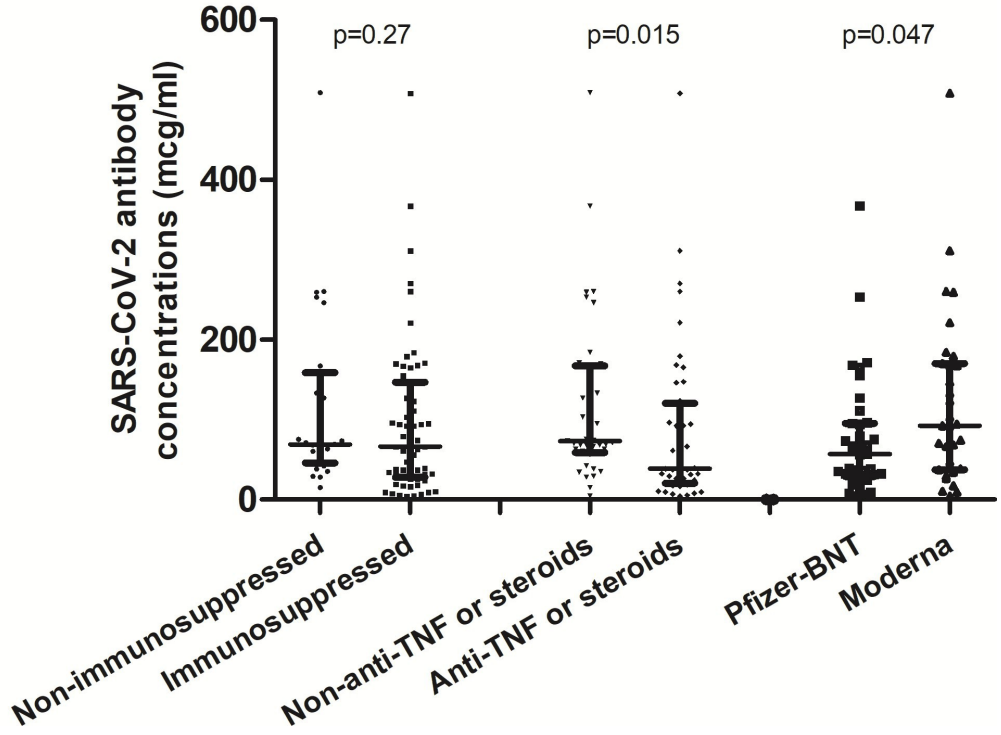

SARS-CoV-2 antibody concentrations (mcg/ml)
600
400
200
0
p=0.27
p=0.015
p=0.047
Non-immunosuppressed
Immunosuppressed
Non-anti-TNF or steroids
Anti-TNF or steroids
Pfizer-BNT
Moderna

Figure 1: A. Serum antibody concentrations in patients with IBD following the two-dose series vs third dose (median 31 (IQR 16–61) vs 68 (IQR 32–147), p<0.001). **B.** Left: serum antibody concentrations following the third dose in patients with IBD on non-immunosuppressive therapy vs immunosuppressive therapy (median 69 (IQR 46–159) vs 66 (IQR 28–147), p=0.27). Non-immunosuppressive therapy was defined as absence of IBD-directed therapy or receipt of treatment with mesalamine monotherapy or vedolizumab monotherapy. Immunosuppressive therapy was defined as thiopurine monotherapy (i.e., azathioprine, 6-mercaptopurine), anti-TNF monotherapy, anti-TNF combination therapy (i.e., plus antimetabolite), ustekinumab monotherapy or combination therapy, tofacitinib, or systemic corticosteroid therapy (i.e., any of the aforementioned groups plus systemic corticosteroids). Middle: subgroup analysis with serum antibody concentrations following the third dose in patients with IBD on “no or less immunosuppression” vs anti-TNF monotherapy, anti-TNF combination therapy, and systemic corticosteroid therapy (73 (IQR 60–167) vs 39 (IQR 20–120), p<0.001). “No or less immunosuppression” was defined as absence of IBD-directed therapy or receipt of mesalamine monotherapy, vedolizumab monotherapy, thiopurine monotherapy, or ustekinumab monotherapy or combination therapy. Tofacitinib was excluded from subgroup analysis due to small sample size. Right: serum antibody concentrations for patients with IBD that received three Moderna doses vs three Pfizer doses (median 94 (IQR 38–170) vs 62 (IQR 31–96), p=0.047).

Chapter 4: Higher Cell-Mediated Immune Responses in Patients with Inflammatory Bowel Disease on Anti-TNF Therapy after COVID 19 Vaccination

Preface

We found that antibody and T-cell responses to COVID-19 vaccines in patients with inflammatory bowel disease do not correlate. Most patients with IBD mount a T cell response despite being on biologic therapies, those on anti-TNF may have a higher T cell response. Anti-TNF therapy has been associated with a lower antibody response to COVID-19 vaccines, but the T cell response is augmented.

Citation

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Therapy after COVID 19 Vaccination

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Abstract

Introduction

Some patients with inflammatory bowel disease (IBD) on immunosuppressive therapies may have a blunted response to certain vaccines, including the mRNA coronavirus disease 2019 (COVID-19) vaccines. However, few studies have evaluated the cell mediated immune response (CMIR), which is critical to host defense after COVID-19 infection. The aim of this study was to evaluate the humoral and CMIR after mRNA COVID-19 vaccination in patients with IBD.

Methods

This prospective study (HERCULES) evaluated humoral and CMIR after completion of two doses of mRNA COVID-19 vaccines in 158 IBD patients and 20 healthy controls (HC). The primary outcome was the CMIR to mRNA COVID-19 vaccines in patients with IBD. The secondary outcomes were a comparison of the (1) CMIR in patients with IBD and HC, (2) CMIR and humoral immune response in all participants and 3) correlation between cell-mediated and humoral immune responses.

Results

The majority (89%) of patients with IBD developed a CMIR which was not different vs HC (94%, p=0.6667). There was no significant difference (p=0.5488) in CMIR response between immunocompetent (median 255 Spike T cells/million PBMC, IQR 146, 958) and immunosuppressed patients (median 377, IQR 123, 1440). There was no correlation between humoral and cell-mediated immunity after vaccination (p=0.5215). In univariable analysis, anti-TNF therapy was associated with a higher CMIR responses (p= 0.02) and confirmed in a multivariable model (p=0.02). No other variables were associated with CMIR.

Discussion

Most patients with IBD achieved CMIR to a COVID-19 vaccine. Future studies are needed evaluating sustained CMIR and clinical outcomes.

Key Messages

- What is already known?

  Most patients with IBD will have an antibody response to COVID-19 vaccines despite being on immune modifying therapies.

- What is new here?

  Most patients with IBD will produce a cell mediated immune response (CMIR) to COVID-19 vaccines. Immune modifying therapies do not appear to blunt CMIR and those on Anti-TNF therapy will have a stronger CMIR.

- How can this study help patient care?

  Our study should reassure providers that immune-modifying therapies used to treat IBD do not appear to affect the CMIR to COVID-19 vaccine, unlike other immunosuppressed populations.

Introduction

Two mRNA coronavirus disease 2019 (COVID-19) vaccines, mRNA-1273 (Moderna) and BNT162b2 (Pfizer-BioNTech), are highly effective in the general population.[1] However the pivotal trials that evaluated the efficacy of these vaccines excluded patients with inflammatory bowel disease (IBD) and other immunosuppressed populations, who may have a lower immune response to selected vaccines.[2-4] These vaccines have been found to be safe in patients with IBD with similar rates of localized and systemic adverse events as found in the general population. Additionally, rates of an IBD flares following vaccination are low (2%). [5, 6] Among immunosuppressed solid organ transplant recipients, seroconversion after COVID-19 vaccines is suboptimal. For example, among 658 transplant recipients, only 54% mounted a humoral immune response after vaccination.[7] In contrast, 95-99% of patients with IBD have measurable

antibody responses after the two-dose mRNA vaccine series.[8-10] However, selected patients have an impaired immune response to the COVID-19 vaccine. The PREVENT-COVID trial observed that lower seroconversion was associated with old age, the BNT162b2 vaccine, and combination therapy with an anti-tumor necrosis factor alpha (TNF)-alpha inhibitor and an immunomodulator.[8] Similarly, a multicenter prospective study from the United Kingdom (UK) found that lower antibody concentrations were associated with age, use of infliximab or tofacitinib , but not ustekinumab, thiopurines or vedolizumab.[11] CLARITY-IBD a large multicenter study in the UK evaluating the impact of infliximab and vedolizumab on humoral immunogenicity of two doses of COVID-19 vaccines found similar rates of seroconversion regardless of treatment (94% (infliximab) and 98% (vedolizumab)). They found that treatment with infliximab, age >60 years, a diagnosis of Crohn's disease, and vaccination with a viral vector vaccine ChAdOx1 was associated with lower antibody concentrations.[12] Other studies have also found that patients with IBD who were vaccinated with non-mRNA vaccines may have lower rates of seroconversion and lower antibody concentrations. [11-14]

The "HumoRal and CellULar initial and Sustained immunogenicity in patients with IBD" (HERCULES) study observed a lower serological response after COVID-19 vaccination in patients with IBD than in healthy controls (HC).[10] However, the clinical relevance of these differences is unknown. It has been shown that antibody concentrations wane with time after vaccination, but cellular immunity may persist.[15] Additionally, many viral variants of concern may evade humoral immunity, but cellular responses induced by vaccines show strong protection against these variants.[16] SARS-CoV-2 specific cellular immune responses are important for viral

clearance, provide robust memory, and mediate recognition of viral variants.[15] Few studies of immune responses to vaccine in patients with IBD have focused on evaluating vaccine induced cell-mediated immune response (CMIR), an important component for protection against viruses such as SARS-CoV2 . The aim of this study was to evaluate the CMIR of COVID-19 vaccine patients with IBD and determine if different immune modifying therapies may impact CMIR.

Methods

This, prospective, non-randomized study (HERCULES) enrolled 158 IBD patients and 20 healthy controls.[10] Participants with IBD were enrolled at the University of Wisconsin-Madison (UW) and Mayo Clinic Florida (MAYOFL). Healthy controls were only enrolled at MAYOFL. Patients with IBD were aged 18-85 years, and on a stable medication regimen in the maintenance phase for at least 2 months, and had completed a mRNA vaccine series.[10] Patients with IBD were categorized into two groups: 1) non systemic immunosuppressive therapy which included: being on no therapy, aminosalicylate monotherapy, or on vedolizumab monotherapy. Vedolizumab was considered in this group since previous studies have shown that it does not appear to impact vaccine responses.[4, 17] The second group was the immunosuppressed group which consisted of being in one of the following treatment groups: thiopurine therapy group: on azathioprine at least 2 mg/kg or 6MP 1 mg/kg; anti-TNF therapy group: on maintenance infliximab (at least every 8 weeks), golimumab (at least monthly), adalimumab (at least every 2 weeks), or certolizumab (at least monthly); anti-TNF combination therapy group: on anti-TNF therapy as described above along with either 15 mg of methotrexate or azathioprine at least 1 mg/kg or 6MP 0.5mg/kg. The anti-TNF therapy group was analyzed together regardless of dosing schedule (standard or accelerated) or type of dose (SQ or IV) given that previous studies have not shown that they

type of therapy impacts vaccine response or if vaccine response was impacted by drug dosing. [18, 19]; ustekinumab therapy group: on either ustekinumab monotherapy or combination therapy with methotrexate or azathioprine; tofacitinib therapy group: on tofacitinib at least 5mg twice daily; and corticosteroid therapy group: on any one of the systemic immunosuppressive groups and any dose of corticosteroids.

Patients with IBD were excluded if they had a previous known diagnosis of COVID-19 infection or had serological evidence of asymptomatic infection. Healthy controls were eligible if they were not on immunosuppressive therapy and had documentation that they completed an mRNA vaccine series. Completion of an mRNA vaccines series was confirmed by review of the Wisconsin Immunization Registry (WIR) for those recruited at UW and via electronic health records for those recruited at MAYOFL. Similar to the original COVID-19 immunogenicity clinical trials , the humoral immune response and CMIR were measured at 28–35 days after the 2-dose mRNA series in patients with IBD and at approximately 30 days in HCs.[20]

Wisconsin Immunization Registry

The WIR is a state-wide database maintained by the Department of Health and Family Services of the State of Wisconsin in which vaccine data for each Wisconsin resident is stored. The WIR captures 97% of vaccines administered in the state, and 98.5% of Wisconsin residents have an active WIR record. The WIR does not capture vaccines administered outside the state, and all Wisconsin vaccine providers are required to enter record of COVID-19 vaccine administration into the registry.[21] The WIR has been previously been used to evaluate COVID-19 vaccine uptake in patients with IBD.[22]

Outcomes

The primary outcome was the CMIR to mRNA COVID-19 vaccines in patients with IBD. The secondary outcomes were a comparison of the (1) CMIR in patients with IBD and HC, (2) CMIR and humoral immune response in all participants and (3) correlation between cell-mediated and humoral immune responses.

Humoral immune response measurements

Nucleocapsid and Spike protein S1 receptor binding domain (RDB)-specific IgG antibodies were measured in sera at 28-35 days post completion of the two dose mRNA series in patients with IBD and at approximately 30 days in HC similarly to COVID-19 immunogenicity clinical trials. [23]

LabCorp's Cov2Quant IgG assay uses electrochemiluminescence immunoassay technology for the quantitative measurement of IgG antibodies to SARS-CoV2. This assay was used to measure the levels of IgG antibodies against S1 receptor binding domain of SARS-CoV-2 (the target of COVID-19 vaccines). Anti-nucleocapsid (indicative of a prior infection) antibodies were measured in all patients with IBD and healthy controls. Anti-nucleocapsid method is qualitative electrochemiluminescence immunoassay by Roche Elecsys platform (Roche Diagnostics, Switzerland). Patients with prior COVID-19 infection (as assessed with a nucleocapsid antibody test) were excluded. The sensitivity and correlation to neutralizing antibodies has been previously described. [10]

Fluorospot Analysis

Fluorospot assays were performed to quantitate antigen-specific T cells capable of secreting interferon-y

(IFN-y) with use of the human IFN-y Fluorospot^{Plus} kit (Mabtech). Cryopreserved peripheral blood mononuclear cells (PBMCs) were thawed at 37°C, washed twice with RPMI media with 10% AB serum (Gemini Bio-Products), and viability determined by trypan blue exclusion using the Cellometer Vision (Nexcelom Bioscience). Only samples with > 85% viability were used in the assay. PMBCs were plated at 2.5×10^5 per well in triplicate in 96-well round bottom plates and incubated at 37 °C, 5% CO^2 for 24 hr. with complete medium alone, Spike protein peptide pools 1 + 2 (Stemcell Technologies, 1µg/ml), or phytohemagglutinin (PHA, 7.5 µg/ml, positive control). The SARS-CoV Spike protein peptides were in separate two pools that consisted of 158 peptides each consisted of 15-mer peptides with 11-amino acid overlaps that span amino acids 1-1273 of the spike protein. After 24 hours, cells were transferred to fluorospot plates pre-coated with anti-IFN-γ and that were blocked for 2 hr. with complete media at 37 °C. Plates were incubated for additional 24 hr., washed, and incubated with biotinylated anti-IFN-y and streptavidin-550 conjugates with washes between each step. After the final wash, plates were incubated for 15 min with fluorescence enhancer-II, and after its removal, dried under a hood blower for 15 min. Plates were read on an AID ELISpot reader (San Diego, CA) using the Cy3 filter. AID Spot parameters were as follows: intensity (min 14; max 250); size min 43, max 5000); emphasis (small) and algorithm C. Antigen-specific T cells were defined as the average number of spots elicited by the antigen of interest minus the average number of spots elicited with culture medium alone. For each patient, the number of Spike-specific T cells was calculated by summing the individual responses to pools 1 and 2. For samples where spots were too numerous to count, spot number was set to 6400. All spot numbers were multiplied by four to achieve a standardized spots per million cells. Six patients with IBD and two healthy controls were excluded in the final analysis due to lack of PHA response. Although the lack of a PHA could indicate profound therapy-induced immune suppression, it could also indicate poor cell quality or lost sample, thus the results were not included. One IBD patient was excluded due to pre-vaccine positive COVID nucleocapsid response.

Data analysis and statistical design

Categorical variables were reported as frequencies (percentages) and continuous variables were reported as median with interquartile range. Mann-Whitney test was used to compare continuous variables between groups and Fisher's exact test was used to compare categorical variables. Spearman's test was used to evaluate for correlations between antibody and T cell responses. Univariable linear regression analysis was conducted to assess the association of CMIR with age, sex and IBD Therapy. Multivariable regression was performed to estimate the relationship between age, anti-TNF therapy, vedolizumab, vaccine type and the CMIR. All tests were two sided with p value < 0.05 considered statistically significant. All analysis were performed using R studio.

Ethical considerations

The study received Institutional Review Board approval at the University of Wisconsin and Mayo Clinic Florida.

Results

A greater proportion of HCs than patients with IBD (85% vs 54%) received the Pfizer vaccine (Table 1). Most patients with IBD had a diagnosis of Crohn's disease (106, 67%), were on stable medication regimens (mean 62 months), and on immunosuppressive therapy (105, 66%).

The spike antibody levels were evaluable in 152 patients with IBD and in 18 healthy controls. A humoral immune response was observed in 97% of patients with IBD vs 100% of HCs. Thus, the numbers of T cells responsive to spike antigens were evaluable in 151 patients with IBD and in 18 HC. Seventeen controls (97%) and one hundred thirty-five patients with IBD (89%) had a CMIR (Table 1, Figure 1A). Three of four participants with no measurable antibodies did have a CMIR

(76, 232, & 4600 Spike T cells/million PBMC). There was no association between levels of antibodies and CMIR (Figure 1B). Among patients with IBD, the humoral but not CMIR response was lower in patients taking vs not taking immunosuppressive medication(s) (Figures 1C-D). Additionally, no difference in Spike T cell responses was found between those on anti-TNF therapy or JAK inhibitors compared to other therapies (Table 2).

In univariable analysis (Supplementary Table 1), anti-TNF therapy was the only variable associated with a higher CMIR, Beta coefficient 594.5 ($p = 0.02$). Age, mRNA vaccine type, or other IBD therapies were not associated with CMIR. In our multivariable model, we confirmed that anti-TNF therapy was associated with higher CMIR (Beta Coefficient 665, $p = 0.02$). Age, vedolizumab or mRNA vaccine type were not associated with CMIR.

Discussion

In this study, essentially all patients with IBD, even those on immunosuppressant medications, mounted a CMIR to the COVID-19 vaccine. By contrast to earlier studies, which observed a lower antibody response after COVID-19 vaccination in immunosuppressed patients with IBD, the CMIR was not significantly different between patients who were vs were not taking immunosuppressants medications. We did not find a correlation between vaccine-induced antibody levels and CMIR, similar to what has been seen in HCs.[16] We did find that anti-TNF therapy was associated with a higher CMIR as was seen in a previous study. [24]

Our findings are in contrast with the impaired cell mediated and humoral responses after COVID-19 vaccination observed in other immunosuppressed populations. For example, a CMIR was observed in 36-46% of solid organ transplant recipients, in 58% of patients on B-cell depleting

therapy, and in 62-74% of patients with psoriasis on biological therapy and/or an immunomodulator.[25-28] The humoral immune response after a primary mRNA series was also impaired in solid organ transplant recipients and in rituximab-treated patients.[7, 26] Studies that have evaluated the CMIR in patients with IBD have found mixed results. In the CLARITY IBD study, the CMIR after the first or second dose of mRNA COVID-19 vaccine was not different between 211 infliximab-treated and 71 vedolizumab- treated patients; up to one fifth of patients did not have a CMIR. They also found a modest positive correlation between T cell responses and antibody concentrations for those who received an mRNA vaccine but no association between T cell responses and antibody concentration in those immunized with ChAdOx1 COVID-19 vaccine (viral vector vaccine).[12] There was no difference observed in T cell response between mRNA and ChAdOx1n COVID-19 vaccines. Among 60 patients with IBD from the Czech Republic, the CMRI, measured twenty-six weeks after 2nd dose with the interferon gamma released assay response was absent in 18% of patients, who were more likely to be on anti-TNF therapy.[29] Similar to CLARITY-IBD, they found an agreement between CMIR and antibody concentrations. In contrast, two other studies observed that most patients with IBD had a measurable CMIR. A small study evaluating CMRI 2 weeks post immunization in 29 patients with IBD found that they had similar frequencies of spike-specific CD4+ and CD8+ T cells, irrespective of their therapy.[30] In the CORRALE IBD study, the T-cell clonal response was observed in all 303 patients with IBD. Compared to those with no treatment, there was no significant effects by ustekinumab, vedolizumab, tofacitinib, or steroids. Those on anti-TNF therapy had an augmented response compared to those on no therapy.[24] These differences among studies may be at least partly explained by differences in the COVID-19 vaccine preparations and the immunization schedules

among studies. For example, UK Health authorities allowed for an extended dosing interval at the beginning of the pandemic so that the second dose of a COVID-19 vaccine could be administered up to 12 weeks later instead of 3 to 4 weeks after the first dose.

A correlation between humoral antibody concentrations and CMIR could be an important finding since unlike antibody tests, evaluating CMIR is an expensive, time-consuming process that is not readily available. The mixed results in the above studies suggest a strong correlation between antibody and CMIR does not exist in patients with IBD. Given the important role of CMIR in viral clearance, immunologic memory and recognition of viral variants, CMIR is a critical component of COVID-19 vaccine-induced protection.[15]

Our results and those of previous studies evaluating antibody responses to COVID-19 vaccines in patients with IBD suggest that most patients with IBD have a vaccine-induced immune response after an mRNA COVID-19 primary series, similarly to healthy controls. An additional dose to the primary series was recommended by the Advisory Committee on Immunization Practices (ACIP) and other international societies for those who are moderately to severely immunocompromised, which included those on anti-TNF therapy, systemic corticosteroids or thiopurines.[31] This recommendation was largely based on evidence that solid organ transplant recipients had a suboptimal rate of seroconversion (56%) after the primary series, and these data were extrapolated to other similarly immunosuppressed populations.[7] This additional dose to the primary series is appropriate for persons who did not mount an adequate initial humoral immune response. [31] Whether an inadequate CMIR also warrants an additional dose to the primary series for most patients with IBD is unknown.[12, 29] After primary immunization, boosters should be administered as recommended for the general population. In fact, studies have shown robust

antibody responses after three doses of COVID-19 vaccines in patients with IBD, with antibody concentrations being higher after the third dose than after the two doses primary series.[32, 33] Such booster doses, preferentially mRNA vaccines, should be given to persons aged 12 years and older, five months after their primary series for the general population and three months in moderately-severely immunosuppressed patients. In late March 2022, the FDA authorized a second booster dose of mRNA-1273 and BNT162b2 for older people and certain immunocompromised individuals at least 4 months after receipt of a first booster. They defined immunocompromised individuals as those who have undergone solid organ transplantation, or who have an equivalent immunocompromised condition.[34] The treatment regimens of most patients with IBD are not equivalent to those of a solid organ transplant recipient. Studies evaluating humoral immunogenicity have found that those on anti-TNF therapy, corticosteroids, and who are older are more likely to have lower antibody concentrations. The clinical relevance of lower antibody concentrations is not known since many of these studies did not have a control group, and it is unknown whether a lower concentration warrants additional doses of COVID-19 vaccines. Based on the FDA most recent guidance, any patients with IBD on anti-TNF therapy, thiopurines, and greater than 20mg of prednisone would be eligible for up to 5 doses of mRNA COVID-19 vaccines.[34]

There are many things we have learned about the use of COVID-19 vaccines such as that immune-modifying therapies other than corticosteroids do not increase the risk of severe COVID-19 disease even prior to widespread vaccination programs.[35] COVID-19 vaccines are safe and not associated with IBD disease flares, and most patients are able to mount a humoral immune response similar to that seen in healthy controls.[5] Additionally, a large population-based study

showed that COVID-19 vaccines are equally effective at preventing infection in patients with IBD compared to non-IBD controls.[36] The goal of COVID-19 vaccines since their inception has been to prevent severe disease that may result in hospitalization, ICU stay or death.[1] This data suggest that most patients with IBD may follow COVID-19 immunization guidelines for the general population rather than for solid organ recipients. Potentially older patients who are on anti-TNF therapy and/or those with risk factors for severe COVID-19 may benefit from 5 mRNA vaccine doses to prevent symptomatic disease. Similarly, while monoclonal antibodies and small molecules are now available to treat COVID-19 disease, most patients with IBD without underlying risk factors for severe disease may not need these therapies. [37]

Our study has several strengths. We evaluated patients on stable treatment regimens. The CMIR was measured with an established assay, the results of which have been associated with protection from disease.[38] We also evaluated CMIR at similar time points of the original COVID-19 vaccine immunogenicity clinical trials. However, there were only 20 controls and a small number of patients with IBD treated with tofacitinib and ustekinumab. We only evaluated one component of the CMIR and did not differentiate between CD4 and CD8 cells. We also only evaluated CMIR after two doses of mRNA vaccines.

In summary, we found that almost all patients with IBD were able to mount CMIR after a two-dose series of a mRNA vaccine which did not correlate with the humoral antibody response. Further studies are needed to evaluate sustained CMIR, the impact of booster doses on CMIR, and long-term antibody concentrations and CMIR in patients with IBD.

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Table 1			
Baseline demographics			
	IBD Patients N=158	**Healthy Controls N= 20**	**P value**
Age in years (median (IQR))	42 (35, 57)	50 (42, 58)	0.2462
Male (%)	79 (50%)	9 (45%)	0.8133
Vaccine Manufacturer			
Moderna	72 (46%)	3 (15%)	0.0086
Pfizer	86 (54%)	17 (85%)	
Type of IBD			
Crohn's Disease (%)	106 (67%)	--	--
Ulcerative Colitis (%)	52 (33%)	--	--
IBD treatment[1]			
Mesalamine monotherapy or no IBD therapy	18 (11%)	--	--
Vedolizumab monotherapy	25 (16%)	--	--
Thiopurine	9 (6%)	--	--
Anti-TNF monotherapy Adalimumab N=33 (10[a]) Infliximab N=28 (11[a])	61 (39%)	--	--
Anti-TNF combination Infliximab n=7 (1[a]) Adalimumab = 6(2[a])	13 (8%)	--	--
Ustekinumab monotherapy or combination	16 (10%)	--	--
Tofacitinib	6 (4%)	--	--
Corticosteroid therapy (2.5-40mg/day)	10 (6%)	--	--

Duration of immunosuppression (mean ± SD)	62.2± 56.7		
Post vaccine immune summary			
Post vaccine Spike antibody concentrations (µg/ml; median and IQR , IBD) (U/ml; median and IQR, Healthy)	34 (17, 67) N=152 evaluable	2500 (1534, 2500) N=20 evaluable	N/A
Post vaccine Spike T cell levels (per million PBMC; media, IQR)	357 (143, 1285) N=151 evaluable	576 (112, 1717) N=18 evaluable	0.3288
Antibody response	147 (97%)	18 (100%)	1.000
Cell-mediated immune response ≥50 spots	130 (89%)	17 (94%)	0.6997

a Dosing of anti-TNF therapy in intensified schedule (e.g. adalimumab more frequent than every 14 days or infliximab more frequent than every 8 weeks).

Table 2 Humoral and Cellular Vaccine Immune Responses

Post vaccine immune responses by IBD therapy or vaccine type			
	Not immunosuppressed[1]	**Immunosuppressed[2]**	**P value (Mann-Whitney U)**
Post vaccine Spike antibody concentrations (µg/ml; median and IQR , IBD)	66 (37,103) N=41 evaluable	27 (14,48) N=111 evaluable	<0.0001
Post vaccine Spike T cell levels (per million PBMC; media, IQR)	255 (146, 958) N=41 evaluable	377 (123, 1440) N=110 evaluable	0.5488
	No anti-TNF or Tofacitinib	**Anti-TNF or Tofacitinib**	**P value (Mann-Whitney U)**

Post vaccine Spike antibody concentrations (µg/ml; median and IQR , IBD)	59 (28, 100) N=74 evaluable	78 (14,38) N=78 evaluable	<0.0001
Post vaccine Spike T cell levels (per million PBMC; media, IQR)	314 (96, 975) N=72 evaluable	401 (172, 1572) N=79 evaluable	P=0.1137
	Pfizer	**Moderna**	**P value (Mann-Whitney U)**
Post vaccine Spike antibody concentrations (µg/ml; median and IQR , IBD)	31 (12, 56) N=81	38 (24, 78) N=71	0.0060
Post vaccine Spike T cell levels (per million PBMC; media, IQR)	380 (146, 1377) N=82	352 (120, 1008) N=69	0.6718
1 Not immunosuppressed= no treatment, mesalamine, budesonide, vedolizumab 2 Immunosuppressed includes all patients treated with thiopurines, anti-TNF, ustekinumab, tofacitinib or corticosteroids.			

Supplementary Table 1. Univariable Analyses of Age, Sex and Therapy Influencing Cell-Mediated Immune Responses to COVID-19 Vaccines		
Demographic	Beta coefficient	P-value
Age	-15.7	0.11
Sex: Male	369.6	0.15
Aminosalicylates Monotherapy	-716.6	0.08
Azathioprine Monotherapy	-508.7	0.23
Methotrexate Monotherapy	219.5	0.75
Anti-TNF therapy	594.5	0.02
Vedolizumab	-303.2	0.37
Ustekinumab	-49.2	0.92
Prednisone	-1004.8	0.051
Tofacitinib	-529.1	0.44

Immunosuppression[1]	388.8	0.18
Moderna Vaccine	101.4	0.69
Post vaccine spike antibody concentrations	-0.65	0.66

[1] Immunosuppression includes all patients treated with thiopurines, anti-TNF, ustekinumab, tofacitinib or corticosteroids.

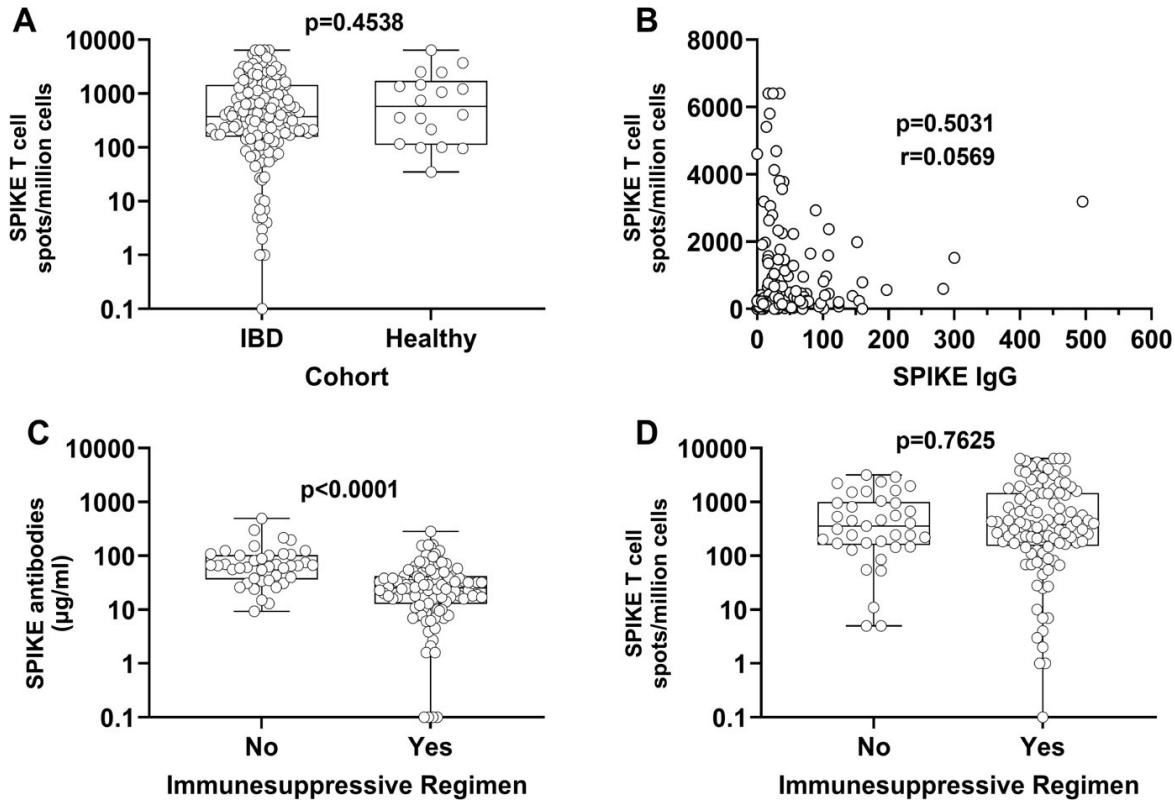

Figure 1. Humoral and cell mediated immune responses in IBD patients and normal healthy individuals following vaccination. ***A***, Box and whisker plot comparing Spike-specific T cell levels (per million PBMC) in all IBD patients and normal healthy controls. P value was calculated using the Mann-Whitney test. ***B*,** Correlation plot comparing paired antibody (µg/ml serum) and Spike-specific T cell (per million PBMC) levels in patients with IBD. P value and r correlation coefficient were calculated using the Spearman correlation test. ***C*-*D***, Box and whisker plot comparing Spike-specific antibody levels (µg/ml serum) and Spike-specific T cell (per million PBMC) levels in IBD patients treated with either non-immunosuppressive or immunosuppressive regimens. P values were calculated using the Mann-Whitney test. Each symbol represents a unique patient or healthy donor.

Chapter 5: Persistence of Antibodies Six Months after Three COVID-19 mRNA Vaccine Doses in Patients with Inflammatory Bowel Disease

Preface

We evaluated the antibody concentrations six months after a third coronavirus disease 2019 messenger RNA vaccine dose in patients with inflammatory bowel diseases. Almost all patients had an antibody response and those with a previous SARS-CoV-2 infection had higher antibody concentrations.

Citation

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Title:
Persistence of Antibodies Six Months after Three COVID-19 mRNA Vaccine Doses in Patients with Inflammatory Bowel Disease

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Keywords:

Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; COVID-19 vaccine

Lay summary

Herein, we evaluated antibody concentrations six months after a third coronavirus disease 2019 messenger RNA vaccine dose in patients with inflammatory bowel diseases. Almost all patients had an antibody response and those with a previous SARS-CoV-2 infection had higher antibody concentrations.

Introduction

Currently four safe and highly efficacious SARS-CoV-2 vaccines have been authorized or fully licensed by the Food and Drug Administration (FDA) in response to the COVID-19 pandemic. Studies evaluating humoral immunogenicity of COVID-19 vaccines in patients with IBD have demonstrated high rates of seroconversion at 95–99% following vaccination with a two-dose mRNA COVID-19 vaccine series and greater than 99% response rate after three doses, which are higher rates of seroconversion than that seen in other immunosuppressed populations such as solid organ transplant recipients.[1-4] Those on anti-tumor necrosis factor (TNF) therapy may have lower antibody concentrations and waning of these antibodies over time.[5] This has raised concerns that patients on anti-TNF therapy may be susceptible to breakthrough cases and/or severe COVID-19.

The Advisory Committee on Immunization Practice (ACIP) currently recommends an additional dose to the primary series for moderately to severely immunocompromised individuals, those who likely did not mount a protective immune response after initial vaccination with the two-dose series. This group includes patients with IBD on high dose corticosteroids (>20mg/day), anti-TNF agents, or immunomodulators.[6] A new bivalent COVID-19 vaccine booster with the ancestral strain of SARS-CoV-2 and Omicron BA.4 and BA.5 is currently recommend for all people ages 5 years and older.[6] To date, there is little evidence of the persistence of antibodies after three doses of COVID-19 vaccine. Thus, the primary aim of this study was to evaluate the sustained antibody concentrations six months after a third COVID-19 mRNA vaccine.

Methods

The HERCULES "HumoRal and CellULar initial and Sustained immunogenicity in patients with IBD" study is prospective study evaluating humoral and cell mediated

immunogenicity of COVID-19 vaccines in patients with IBD. Methods and inclusion criteria for HERCULES have been described previously.[4] Here, we analyzed antibody concentrations in serum samples available at one month (28–65 days) (t_1) and six months (+/-45 days) (t_2) after a third dose of an mRNA COVID-19 vaccine. A small cohort had blood available one month 28-65 days (t_3) after a fourth dose of an mRNA vaccine.

The primary outcome was total serum SARS-CoV-2 anti-spike IgG antibody concentrations at (t_2) compared to (t_1). Secondary outcomes included evaluating the effects of vaccine manufacturer, immune modifying therapies on sustained antibody concentrations, previous history of COVID-19 infection (was defined by a molecular-confirmed diagnosis of SARS-CoV-2 infection via PCR after (t_1) or nucleocapsid antibody seroconversion at (t_2)), and antibody concentration at (t_3) compared to (t_2). Specific antibodies measured in sera using ELISAs performed at Labcorp were nucleocapsid and spike protein S1 receptor-binding domain (RDB)-specific IgG antibodies reported as mcg/ml, as previously described at every timepoint.[2] An additional secondary objective was to determine how many individuals develop a COVID-19 infection after (t_2) till last follow up date (September-November 2022).

Antibody concentrations between groups were compared using Mann-Whitney U tests. Univariable linear regression analysis was conducted to assess the association of antibody concentrations at (t_2) with age, sex, IBD Therapy and previous infection. Multivariable regression was performed to estimate the relationship between age, anti-TNF therapy, vedolizumab, vaccine type and antibody concentrations. All tests were two sided with p value < 0.05 considered statistically significant. All analysis were performed using SPSS V27 or R studio 4.21.

The study received IRB approval at the University of Wisconsin-Madison School of Medicine and Public Health.

Results:

One hundred eight patients had antibody concentrations measured at (t_1), and 164 at (t_2) and 49 at (t_3).(Table 1) The median time since third dose at (t_2) was 170 days (interquartile range (IQR) 166-177). The majority of individuals (90.2%) received a third dose that was the same as their original *two dose* primary series. Almost all individuals 162/164 (98.7%) had measurable antibodies at (t_2), and all 49 patients had measurable antibodies at (t_3). Thirty six (22%) individuals at t_2 had a previous COVID-19 infection. Nineteen individuals developed a COVID-19 infections after (t_2) when followed till last study date. These individuals had similar antibody concentrations at (t_2) (n=19, 22 µg/ml (IQR 11-61)) compared to those never developed an infection (n= 112, 26 µg/ml (IQR 19-47), p =0.88).

Antibody concentrations were significantly lower at (t_2) 31 µg/ml (IQR 11-107) compared to at (t_1) 77 µg/ml (IQR 35-168); p<0.001). Those on anti-TNF therapy were more likely to have lower antibody concentrations (median (IQR) 20 µg/ml (8-62) compared to those on other IBD therapies (median (IQR) 35 µg/ml (18-142) p=0.001). Those with evidence of a previous COVID-19 infection had higher antibody concentrations (median (IQR) 189 µg/ml (65-304) vs. 24 µg/ml (9-47) p<0.001). (Figure 1) In univariable analysis, prior COVID infection was the only variable associated with higher antibody concentrations (beta coefficient = 179.5, p <0.001). In our multivariable model, we confirmed that prior COVID infection was associated with higher antibodies (beta coefficient = 179.2, p <0.001). Age, sex, anti-TNF therapy, or vedolizumab was not associated with higher or lower antibody concentrations.

In univariable analysis excluding the individuals with a previous COVID infection those on anti-TNF or steroids (n = 61, beta coefficient = -44.3, p < 0.025) were more likely to have lower antibody concentrations. In a multivariable model excluding those with prior COVID

infection, those on anti-TNF or steroids (beta coefficient = -67.2, $p < 0.0026$), were more likely to have lower antibody concentrations. Age, sex, vedolizumab or ustekinumab was not associated with higher or lower antibody concentrations.

Antibody concentrations at (t_3) who in those received a fourth dose of a COVID-19 vaccine (n=49; median 98 µg/ml; IQR 36-214 had higher antibody concentrations compared to those at (t_2). concentration (n=164; median 31 µg/ml; IQR 11-107; $p<0.001$).

Discussion

Almost all patients with IBD had a sustained humoral immune response six months after a third dose of an mRNA COVID-19 vaccine. To our knowledge, we are one of the few groups to report antibody concentrations after a fourth dose of a COVID-19 vaccine where all patients with IBD had a robust humoral immune response. We found that those with a previous COVID infection had higher antibody concentrations than those without an infection which has been seen in other populations.[7] Similar to other studies we found that those on anti-TNF therapy or steroids were more likely to have lower antibody concentrations compared to those not on anti-TNF therapy.[3, 4]

Three studies (HERCULES, PREVENT-COVID, & VIP (Vaccination Immunogenicity against SARS-CoV2 in Patients with inflammatory bowel disease)) have evaluated humoral immune responses after a third dose of COVID-19 vaccine typically within 2 months after vaccination and have shown significant boosting of the humoral immune response compared to after the two dose primary series. [2, 3, 8] In PREVENT-COVID and VIP patients on tofacitinib or anti-TNF combination therapy with an immunomodulator were more likely to have lower antibody concentrations.[2, 8] In contrast, in a recent Canadian study and in our HERCULES cohort being on anti-TNF therapy was not associated with a significant reduction in spike

antibody concentrations, only prednisone use at the time of vaccination was associated with lower antibody concentrations.[3, 9] STOP COVID also found that antibodies decay by 12% per week after a third dose. They evaluated antibody concentrations at least one week following a third dose and up to greater than 100 days post vaccination. The majority of their cohort had blood collected approximately two months after the third dose with only 9 patients having their blood drawn after 100 days. We found that the majority of our cohort still had a sustained humoral response with a median time of 170 days after their third dose showing that the robust response after three doses persisted. Additionally, we showed that a previous COVID-19 infection boosted humoral immune responses regardless of IBD treatment regimen. We also showed that a 4th dose of a COVID-19 is able to significantly boost the humoral immune response after a third dose and all patients had a measurable immune response. A recent updated report from STOP COVID also showed robust antibody responses after a 4th dose of a COVID-19 vaccine. [10] When they evaluated sustained antibody concentrations after a third dose individuals older than 65, people only corticosteroids, anti-TNF therapy or combination therapy were more likely to have lower antibody concentrations. [10] They concluded that these groups would most likely benefit from a fourth dose. These studies should provide reassurance to gastroenterology providers that most patients with IBD on immune modifying therapies mount vastly different COVID-19 vaccine-induced immune response compared to solid organ transplant recipients who have significantly lower rates of seroconversion or sustained antibody concentrations after a third dose.[1]

The ACIP has provided new recommendations regarding COVID-19 vaccine use for the Fall 2022 season. People ages 5 years and older should receive one age-appropriate bivalent mRNA booster dose after completing a primary series, and this recommendation replaces all prior booster recommendations. The booster recommendation is the same for general population and for people who are considered moderately or severely immunocompromised.

The bivalent booster is recommended 3 months after a COVID infection or at least 2 months after the last ancestral only booster dose.[6] Thus, gastroenterology providers should strongly recommend a new bivalent COVID-19 booster to all their patients with IBD.

Our study has several strengths. We evaluated sustained humoral immunogenicity in patients on stable medication regimens which permitted us to assess the effect of medications on the immune response to vaccination. We also included a small cohort evaluating immunogenicity after the fourth dose. Our study is limited in its sample size, small representation of certain treatment regimens, the absence of a reference HC population, and not evaluating cell-mediated immune response (CMIR) after a COVID-19 vaccine in this cohort. We previously found that CMIR is uncoupled from the humoral immune response.[11]

In conclusion, almost all patients with IBD had a sustained humoral immune response six months after a third dose of a COVID-19 mRNA vaccine dose. Further studies are needed to investigate the benefit of the upcoming bivalent booster doses.

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Table 1 Baseline Demographics			
Characteristics	**IBD Subjects 1-2 months post 3rd dose (n=180)** [b]	**IBD Subjects 6 months post 3rd dose (n=164)**[c]	**P value**
Demographics			
Age, years, median (IQR)	44.5 (35-59)	43.5 (36 - 59)	0.99
Male, n (%)	90 (50%)	85 (52%)	0.75
Type of IBD			
Crohn's disease, n (%)	108 (60%)	97 (59%)	0.99
Ulcerative colitis, n (%)	69 (38%)	64 (39%)	
IBD unclassified, n (%)	3 (2%)	3 (2%)	
Vaccine data			
Dose 3 type different than original, n (%)	14 (8%)	16 (10%)	0.52
Time between completion of 2-dose series and third dose, days, median (IQR)	194 (150 – 222)	188.5 (147 – 216)	0.44
IBD treatment			
Nonsystemic immunosuppression,[d] n (%)	61 (34%)	55 (34%)	0.94
Aminosalicylate or no IBD therapy, n (%)	29 (16%)	27 (17%)	0.93
Vedolizumab monotherapy, n (%)	32 (18%)	28 (17%)	0.86
Duration of therapy,[e] months, median (IQR)	24 (11-53)	24 (11– 57)	0.74
Systemic immunosuppression,[f] n (%)	119 (66%)	109 (67%)	0.94
Thiopurine monotherapy, n (%)	13 (7%)	13 (8%)	0.80
Anti-TNF monotherapy, n (%)	57 (32%)	48 (29%)	0.63
Anti-TNF combination therapy, n (%)	21 (12%)	22 (13%)	0.62
Ustekinumab monotherapy or combination therapy, n (%)	23 (13%)	21 (13%)	0.99
	5 (3%)	5 (3%)	0.88
Tofacitinib monotherapy, n (%)	9 (5%)	12 (7%)	0.37
Systemic corticosteroid therapy, n (%)	43 (18 - 83)	40 (18 -84)	0.99
Duration of therapy, months, median (IQR)			

Serum antibody concentrations			
Detectable antibody concentrations, n (%)	177 (98%)	162 (99%)	0.73
Serum antibody concentrations, mg/mL, median (IQR)	77 (35-168)	31 (11-107)	<0.001
Time between vaccine dose and antibody measurement, days, median (IQR)	41.5 (35 – 50)	170 (166 – 177)	
6 months post dose antibody concentrations (mg/ml)			
	No	Yes	
Anti-TNF agent therapy	35 (18-142) N=94	20 (8-62) N=70	0.001
Immunosuppressed (AZA, anti-TNF, steroid)	36 (21-147) N=72	23 (8-65) N=92	0.001
Immunosuppressed (AZA, anti-TNF, ustekinumab, tofa, steroid)	52 (25-189) N=53	25 (9-63) N=111	<0.001
Previous COVID-19 infection	24 (9-47) N=128	189 (65-304) N=36	<0.001

Abbreviations: IBD, inflammatory bowel disease; IQR, interquartile range; TNF, tumor necrosis factor.

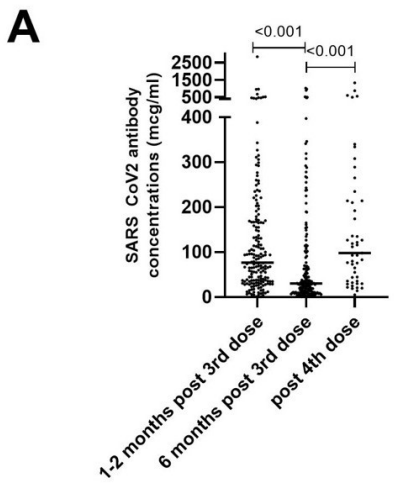
[b] Completed third dose and had serum antibody concentrations measured *** days thereafter.

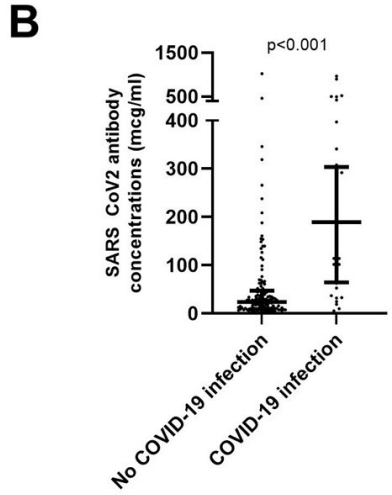
[c] Completed third dose and had serum antibody concentrations measured *** days thereafter.

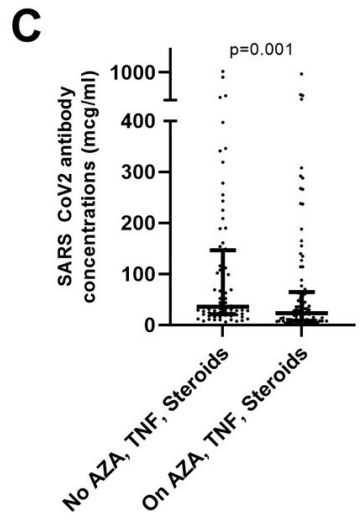
[d] Absence of IBD therapy, mesalamine monotherapy, or vedolizumab monotherapy.

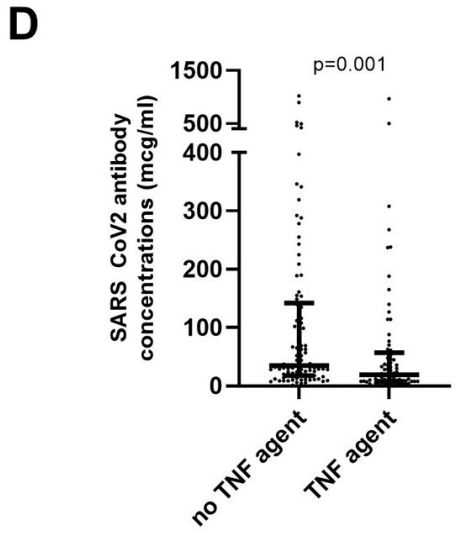
[e] Subjects with an absence of IBD therapy were omitted from the calculation of the duration of therapy.

[f] Thiopurine monotherapy (ie, azathioprine, mercaptopurine), anti-TNF monotherapy, anti-TNF combination therapy (ie, plus antimetabolite), ustekinumab monotherapy or combination therapy, tofacitinib, or systemic corticosteroid therapy (ie, any of the aforementioned groups plus systemic corticosteroid).


A
<0.001
<0.001
SARS CoV2 antibody concentrations (mcg/ml)
2500
1500
500
400
300
200
100
0
1-2 months post 3rd dose
6 months post 3rd dose
post 4th dose


B
p<0.001
SARS CoV2 antibody concentrations (mcg/ml)
1500
500
400
300
200
100
0
No COVID-19 infection
COVID-19 infection


C
p=0.001
SARS CoV2 antibody concentrations (mcg/ml)
1000
400
300
200
100
0
No AZA, TNF, Steroids
On AZA, TNF, Steroids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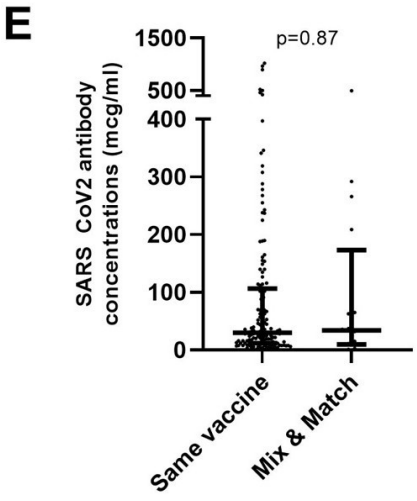
p=0.001
SARS CoV2 antibody concentrations (mcg/ml)
1500
500
400
300
200
100
0
no TNF agent
TNF agent


E
p=0.87
SARS CoV2 antibody concentrations (mcg/ml)
1500
500
400
300
200
100
0
Same vaccine
Mix & Match

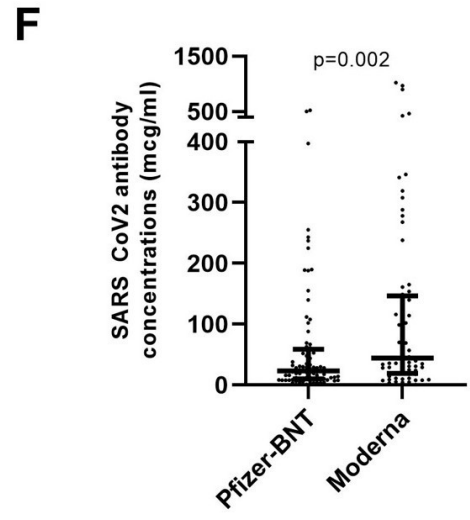

F
p=0.002
SARS CoV2 antibody concentrations (mcg/ml)
1500
500
400
300
200
100
0
Pfizer-BNT
Moderna

Figure 1 Antibody concentration in patients with inflammatory bowel disease six months post third dose

1A) Antibody concentrations at 1-2 months post third dose, six months post third dose and 1 month post 4th dose.
1B) Antibody concentrations in patients with a prior COVID-19 infection compared to those without a COVID-19 infection.
1C) Antibody concentrations in patients on azathioprine, anti-TNF, and steroids compared to those not on those therapies.
1D) Antibody concentrations in patients on anti-TNF therapy compared to those not on anti-TNF therapy
1E) Antibody concentrations in patients who received a third dose that was the same as their original two dose primary series compared to those who did a mix and match strategy (different third dose than primary series).
1F) Antibody concentrations in patients who received a Pfizer-BioNTech vs a Moderna vaccine as third dose.

Chapter 6: Higher and Sustained Cell-Mediated Immune Responses after Three Doses of mRNA COVID-19 Vaccine In Patients with Inflammatory Bowel Disease on Anti-TNF Therapy

Preface

All patients with inflammatory bowel disease despite being on biologic therapies mount a T cell response after a third dose of a coronavirus disease 2019 messenger RNA vaccine. The response is maintained after six months and those on anti-tumor necrosis factor may have a higher T cell response. Antibody and T cell response after a third dose do not correlate.

Citation

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Title: Higher and Sustained Cell-Mediated Immune Responses after Three Doses of mRNA COVID-19 Vaccine In Patients with Inflammatory Bowel Disease on Anti-TNF Therapy

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Keywords: Crohn’s disease, Ulcerative colitis, immunology, immune response.

Abstract

Introduction: Studies suggest that the generation of durable T cell immunity following COVID-19 vaccination protects against severe disease. The aim of this study was to measure cell mediated immune response (CMIR) one to two months and six months after a third dose of a COVID-19 mRNA vaccine.

Methods: This prospective study (HERCULES) evaluated CMIR at 28–65 days (t_1) after dose 2, 28–65 days (t_2) (n=183) and six months (+/-45 days) (t_3) (n=167) after a third dose of an mRNA COVID-19 vaccine. A small cohort had blood available 28-65 days (t_4) (n=55) after a fourth dose. Primary outcomes were CMIR at (t_2) and (t_3). Secondary outcomes included the effect of immunosuppressing IBD medications on CMIR and response at (t_4).

Results: All patients had measurable CMIR at all time points. CMIR increased at t_2 compared to t_1 (median 1467 responding cells per million (interquartile range (IQR) 410-5971) vs 313 (94-960) $p< 0.001$). There was no significant waning when comparing t_2 vs t_3 or significant boosting at t_4. Those on anti-TNF monotherapy had a higher CMIR compared to those not on this therapy at t_2 (4132 (IQR 1136-8795) vs. 869 (IQR 343-3221) $p <0.001$) and t_3 (2843 (IQR 596-6459) vs 654 (IQR 143-2067) $p<0.001$). In univariable analysis, anti-TNF monotherapy was associated with a higher CMIR at t_2 ($p< 0.001$) and t_3 ($p< 0.001$) and confirmed in a multivariable model ($p< 0.001$).

Conclusion: A third dose of a COVID-19 vaccine boosts CMIR, and the response is sustained in patients with IBD.

Study Highlights

- **WHAT IS KNOWN**

  Patients with inflammatory bowel disease mount a cell mediated immune response (CMIR) after a second and third dose of COVID-19 vaccine.

- **WHAT IS NEW HERE**

  A third dose of a COVID-19 vaccine boosts CMIR and the response is sustained for six months. There is no significant boosting after a fourth dose. Those on anti-TNF monotherapy have an augmented response compared to those not on anti-TNF monotherapy.

- **TRANSLATIONAL IMPACT**

  The mechanism of anti-TNF monotherapy augmenting COVID-19 vaccine response should be explored further.

Introduction

Four coronavirus disease 2019 (COVID-19) vaccines, mRNA-1273 (Moderna), BNT162b2 (Pfizer-BioNTech), Ad.26.COV2.S (Johnson & Johnson; no longer available in U.S.), and NVX-CoV2373 (Novavax) have been found to be highly effective in the general population.(1, 2) The original clinical trials evaluating the efficacy of these vaccines excluded patients with inflammatory bowel disease (IBD) and other immunosuppressed populations, who historically may have a lower immune response to non-COVID-19 vaccines.(3, 4) COVID-19 vaccines have been found to be safe in patients with IBD with similar rates of localized and systemic adverse events as found in the general population.(5, 6) Additionally, COVID-19 vaccination is not associated with IBD flares with low rates reported in prospective studies.(6)

Multiple studies have evaluated humoral immunogenicity of COVID-19 vaccines and found that most patients with IBD are able to mount measurable antibody responses after the two-dose mRNA vaccine series (95-99%).(7-12) Robust humoral responses are seen after three doses with greater than 99% response rate, which are higher rates of seroconversion than that seen in other immunosuppressed populations such as solid organ transplant recipients.(13-15) Furthermore, patients with IBD have a sustained humoral immune response to COVID-19 vaccines with the majority of patients having measurable antibodies six months after a third dose of a COVID-19 vaccine.(16) However, those on anti-tumor necrosis factor (TNF) therapy may have lower antibody concentrations after two or three doses of COVID-19 vaccines, with faster waning of antibodies.(12) Those with waning humoral immunity may be more susceptible to breakthrough infections and boosting by additional doses are protective.(17) While antibody concentrations wane with time after vaccination, cellular immunity may

persist.(18) SARS-CoV-2 specific cellular immune responses provide robust memory, mediate recognition of viral variants, and are important for viral clearance.(18) Additionally, many viral variants of concern may evade humoral immunity, but cellular responses induced by vaccines show strong protection against these variants.(19) Thus, evaluating the COVID-19 vaccine induced cell mediated immune response (CMIR) in patients with IBD on immune modifying therapy is of utmost importance.

In contrast to vaccine-induced humoral immunity, fewer studies have evaluated vaccine-induced CMIR in patients with IBD. Initial studies found that most patients develop CMIR after the mRNA primary series.(9, 20, 21) Two studies found that those on anti-TNF therapy have a higher CMIR compared to those on non-TNF biologics or immunomodulators after two doses of vaccine.(20, 21) Two studies that evaluated CMIR immediately after a third dose of COVID-19 vaccine found that those on anti-TNF therapy mounted similar[10] or lower CMIR*(22)* compared to healthy controls*.* To further evaluate the CMIR after COVID-19 vaccination, the primary aim of the present study was to measure CMIR at one to two months and approximately six months after a third dose of mRNA COVID-19 vaccine. Secondary aims were to evaluate the impact of a fourth dose; impact of immune-modifying therapies; effects of homologous or heterologous boosting; and prior COVID-19 infection on CMIR. We also aimed to examine the correlation between CMIR and humoral immune responses. We hypothesized that those on anti-TNF therapy would continue to demonstrate higher CMIR compared to patients with IBD on other treatments as we have previously seen in our HERCULES cohort.

Methods

Study Design

The HERCULES "HumoRal and CellULar initial and Sustained immunogenicity in patients with IBD" study is a prospective study evaluating humoral responses and CMIR of COVID-19 vaccines in patients with IBD. Participants with IBD were enrolled at the University of Wisconsin–Madison (UW). Methods and inclusion criteria for HERCULES have been described previously.(8) Briefly, eligibility criteria were a diagnosis of IBD, ages 18-85 years, on stable doses of maintenance therapy at least two months, and three mRNA vaccine doses. Patients were divided into the in the following groups. The non-systemic immunosuppressive group:on mesalamine monotherapy or no therapy for IBD, or on vedolizumab monotherapy. Vedolizumab was considered in this group since previous studies have shown that it does not appear to impact vaccine response (4, 23). The immunosuppressed group consisted on being one of the following groups: thiopurine therapy group: on azathioprine at least 2 mg/kg or 6MP 1 mg/kg; anti-TNF therapy group: on maintenance therapy infliximab (at least every 8 weeks), golimumab (at least monthly), adalimumab (at least every 2 weeks), or certolizumab (at least monthly); anti-TNF combination therapy group: on anti-TNF therapy as described above along with either 15 mg of methotrexate or azathioprine at least 1 mg/kg or 6MP 0.5mg/kg; ustekinumab therapy group: on either ustekinumab monotherapy or combination therapy with methotrexate or azathioprine; tofacitinib therapy group: on tofacitinib at least 5mg PO BID and ; corticosteroid therapy group: on any one of the systemic immunosuppressive groups and any dose of corticosteroids.

We previously reported the CMIR at 28–65 days (t_1) after the two dose primary series.(21) Here, we analyzed CMIR in lymphocytes available at 28–65 days (t_2) and approximately six months (+/-45 days) (t_3) after a third dose of an mRNA COVID-19 vaccine. A small cohort had blood

available 28-65 days (t_4) after a fourth dose of an mRNA vaccine. Samples were analyzed from individuals who were not lost to follow up from the original cohort, had not changed treatment since the initial two dose series, and had received a third or fourth dose of a COVID-19 vaccine. Change in treatment was defined as changing in treatment group (e.g. from anti-TNF therapy to ustekinumab therapy). A change in dose or addition of an immunomodulator to a participant on a biologic was not considered a change in therapy. There were participants who changed therapy between t2 and t3 and these were excluded.

Completion of an mRNA vaccines series was confirmed by review of the Wisconsin Immunization Registry (WIR). A previous history of COVID-19 infection was defined by a molecular-confirmed diagnosis of SARS-CoV-2 infection via PCR confirmed in chart review or nucleocapsid antibody seroconversion after 28–65 days (t_2) or approximately six months (+/-45 days) (t_3) after a third dose. All patients signed informed consent. The study received IRB approval at the University of Wisconsin-Madison School of Medicine and Public Health.

Wisconsin Immunization Registry

The WIR is a state-wide database maintained by the Department of Health and Family Services of the State of Wisconsin in which vaccine data for each Wisconsin resident is stored. The WIR captures 97% of vaccines administered in the state, and 98.5% of Wisconsin residents have an active WIR record. The WIR does not capture vaccines administered outside the state, and all Wisconsin vaccine providers are required to enter record of COVID-19 vaccine administration into the registry.(24) The WIR has been previously used to evaluate COVID-19 vaccine uptake in patients with IBD.(25)

Outcomes

The primary outcomes of this study were CMIR at one to two months (28–65 days) (t_2) and approximately six months (+/-45 days) (t_3) after a third dose of an mRNA COVID-19 vaccine. We chose these time points to evaluate CMIR immediately after immunization (t_2) the same time point used when we evaluated humoral and CMIR after a two dose series and sustained immunogenicity at six months since that is typical time that has been used to evaluate sustained immunogenicity of vaccines in other studies. (4) (26)

Secondary outcomes included CMIR at 28-65 days (t_4) after a fourth dose, evaluating the effects of homologous boosting (receiving same mRNA vaccine as primary series) as compared to heterologous boosting (different from the primary vaccine), effects of immune modifying therapies on CMIR, particularly anti-TNF monotherapy, anti-TNF combination therapy with an immunomodulator, and previous history of COVID-19 infection (as defined above), and correlations between CMIR and humoral immune responses.

Humoral immune response measurement

Nucleocapsid and spike protein S1 receptor binding domain–specific antibodies were measured in sera at 28–65 days (t_2) and approximately six months (+/-45 days) (t_3) after a third dose of an mRNA COVID-19 vaccine, and at 28-65 days (t_4) after a fourth dose. Specific antibodies measured in sera using electrochemiluminescent immunoassays (ECLIA) performed at LabCorp were anti-nucleocapsid and anti-spike S1 receptor-binding domain (RBD)-specific IgG antibodies reported as U/mL and mcg/ml respectively, as previously described at every timepoint.(8)

Cell-mediated immune response (CMIR) measurements

Fluorospot assays were performed to quantitate antigen-specific T cells capable of secreting interferon (IFN)-γ with use of the human IFN- γ FluorospotPlus kit (Mabtech) from samples at (t_2), (t_3), and at (t_4) as previously described.(21) Antigen-specific T cells were enumerated using spike protein peptide pools 1 + 2 (Stemcell Technologies, 1μg/ml), which are pools of 158 peptides each consisting of 15-mer peptides with 11-amino acid overlaps that span amino acids 1-1273 of the spike protein.

Data Collection

The following clinical variables were collected for participants. At the baseline visit a medical history was collected to determine their IBD history, IBD medications, and immunization history. At the subsequent visit patients were asked about their IBD medications, any change in therapy, and timing of COVID-19 vaccine boosters. Furthermore, research coordinators reviewed the medical record for any change in the IBD therapy at follow up visits.

Statistical analysis

CMIR between groups or time points were compared using Mann-Whitney U and Wilcoxon signed rank tests, respectively. Univariable and multivariable linear regression analysis was conducted to assess the association of CMIR at 28–65 days after dose three (t_2) and at approximately six months (+/-45 days) after dose three (t_3) with age, sex, mRNA vaccine type, homologous or heterologous boosting, previous COVID infection, and IBD therapy (aminosalicylate monotherapy, thiopurine monotherapy, anti-TNF therapy, vedolizumab,

ustekinumab, systemic corticosteroids, or tofacitinib). To evaluate the association between CMIR and these variables we used a multivariable linear regression model with variable that had a p value <0.05. The residuals had constant variance and normally distributed. Multivariable linear regression was performed to estimate the relationship among age, anti-TNF therapy, aminosalicylates, and the CMIR. All tests were two-sided with p value < 0.05 considered statistically significant. All analysis were performed using SPSS V27 or R studio 4.2.1.

Results

One hundred eighty-three patients had CMIR measured at 28–65 days after dose three (t_2), 167 at approximately six months (+/-45 days) after dose three (t_3) and 55 at 28-65 days after a fourth dose (t_4) (Table 1 & 2). The median time blood was collected at 28–65 days after dose three (t_2) was 42 days (interquartile range (IQR) 35-51), median at approximately six months (+/-45 days) after dose three (t_3) of blood collection was 168 days (IQR 147-175). The majority of individuals (169/183, 92%) received a homologous third dose that was the same as their original two dose primary series. The majority of individuals who received a fourth dose (52/55,95%) also received the same as the primary series (Table 2). We previously reported that 89% of individuals at 28–65 days (t_1) after dose 2 had a CMIR.[20] All patients after three doses at (t_2) & (t_3) and after a fourth dose (t_4) had a measurable CMIR. Thirty-eight (23%) individuals at approximately six months (+/-45 days) after dose three (t_3) had a previous COVID-19 infection compared to 16 (9%) at 28-65 days after the third dose (p<0.001).

Cell mediated immune response post third dose (t_2 and t_3)

CMIR increased at (t_2) compared to after the primary two dose series (t_1) (t_2 median 1444

responding cells/million (interquartile range (IQR) 421-6011) vs t_1 median 313 responding cells/million (94-960) p< 0.001(8)), (Figure 1A). Waning of CMIR over the 6-month interval following dose 3 was not statistically significant (n=161 pairs with samples at both timepoints; t_3 median 1241 cells/million (IQR 301-4609) Wilcoxon signed rank p=0.071). No significant boosting was seen at t_4 (t_4 median 1387 responding cells/million (IQR 203-3843) p=0.88). Those on anti-TNF monotherapy had a higher CMIR compared to those not on this therapy at t_2 (median 3927 (IQR 1094-8619) vs. 853 (IQR 343-2984) Mann-Whitney U test p <0.001) and t_3 (median 2876 (IQR 614-7094) vs 671 (IQR 161-2191) p<0.001). CMIR waning was not statistically significant in either the TNF agent group (t_2 vs t_3 Wilcoxon signed rank p=0.20) nor the non-TNF agent group (t_2 vs t_3 p=0.18) (Figure 1B). At (t_3), history of COVID-19 infection did not affect CMIR (no COVID-19 infection median 1024 responding cells/million; IQR 228-4609 vs COVID-19 infection history median 1690 responding cells/million; IQR 311-3913; p=0.098) (Figure 1C). No correlation between antibodies concentrations and CMIR was found at any timepoint. (Figure 1D) The number of participants who received heterologous vaccine series was insufficient to test the hypothesis that CMIR would be different between homologous and heterologous series (homologous series: n=155; median 1271 responding cells/million; IQR 305-4280 vs heterologous series: n=12; median 472 responding cells/million; IQR 56-5253; p=0.30).

Univariable and multivariable analyses at 28-65 days post third dose (t_2)

In univariable analysis evaluating CMIR at (t_2), increasing age (beta coefficient (ß = -51.16, p=0.009), being on aminosalicylates therapy (ß = -1517.9, p =0.046), or on systemic corticosteroids (ß = -2805.9, p=0.035) were associated with a lower CMIR. Being male (ß = 1199.4, p=0.037) and being on anti-TNF monotherapy were associated with a higher CMIR (ß = 2627.4, p <0.001). Those on anti-TNF combination therapy did not have higher or lower CMIR

(Table 3). In our multivariable model, we confirmed that being on anti-TNF monotherapy was associated with a higher CMIR (ß = 2056.9, p <0.001). Being male was also associated with higher CMIR (ß = 1152.4, p <0.036). No other variables were associated higher or lower CMIR in the multivariable model (Table 3).

Univariable and multivariable analysis at approximately six-months post third dose (t_3) .

In univariable analysis evaluating CMIR at (t_3), increasing age (beta coefficient (ß = -46.5, p= 0.02) or being on aminosalicylates therapy (ß = -1752.8, p =0.024) was associated with a lower CMIR, while being on anti-TNF monotherapy was associated with a higher CMIR (ß = 2469.9, p < 0.001). Those on anti-TNF combination therapy did not have higher or lower CMIR (Table 4). In our multivariable model, we confirmed that being on anti-TNF monotherapy was associated with a higher CMIR (ß = 2089.3, p < 0.001). No other variables were associated with a lower or higher CMIR in the multivariable model (Table 4).

Discussion

We evaluated CMIR at approximately one to two months and six months after a third mRNA COVID-19 vaccine and found that all patients with IBD regardless of their treatment regimen had measurable responses. We compared CMIR after two doses of vaccine (89% in HERCULES study) to CMIR after three doses of vaccine, after which 100% of study participants had measurable CMIR, demonstrating benefit of a third dose of mRNA vaccine.(21) This has not been evaluated in previous studies in the IBD population. Additionally, our study did not show waning of CMIR at six months post 3-dose series. Those on anti-TNF monotherapy had higher CMIR at both times points after the third dose compared to those not on anti-TNF therapy or those on anti-TNF combination therapy with an immunomodulator, which is similar to what we saw after two doses of mRNA vaccine.(21) Furthermore, those on anti-TNF therapy did

not have significant waning of CMIR at six months. These findings can reassure patients and providers that even among patients with IBD on immune modifying therapies such as anti-TNF therapy, immune responses to the vaccine are robust and durable. This is also, to our knowledge, the only study to examine CMIR after a fourth dose of vaccine. Although this subset of patients was smaller, we failed to show a boost in CMIR with a fourth dose of vaccine.

Two previous studies have evaluated CMIR after a third dose of the COVID-19 vaccine.(10, 22) The VIP (COVID-19 vaccine-induced antibody and T-cell responses in immunosuppressed patients with IBD after the third vaccine dose) study examined antigen-specific T-cell responses 28-49 days after the third vaccine dose.[10] The majority of patients with IBD (191/202; 95%) mounted a CMIR.. CMIR was similar among COVID-19 infection-naïve patients with IBD on thiopurines, infliximab, thiopurine plus infliximab, ustekinumab, or vedolizumab, compared to healthy controls. Infection-naïve patients receiving tofacitinib had lower CMIR compared to healthy controls. In those with previous SARS-CoV-2 infection, CMIR against SARS-CoV-2 nucleocapsid mapped epitope pool was significantly lower in patients treated with ustekinumab than in healthy controls.

The systemic and T cell-associated responses to SARS-CoV-2 immunization in gut inflammation (STAR SIGN) study evaluated humoral responses and CMIR 2-16 weeks after receiving the third mRNA vaccine dose among 139 patients on biologics and 110 healthy controls.(22) In contrast to the VIP and the present study, a significantly higher proportion of those on anti-TNF therapy (15/73, 20.5%) failed to mount an adequate CMIR to the third dose of vaccine, compared to healthy controls (2/100, 2%) as measured by an interferon-y release assay. There was no significant difference between CMIR in anti-TNF treated and non-anti-TNF treated patients with IBD. The VIP study and our study each used ELIspot or Fluorospot to measure CMIR which has been shown to detect CMIR in immunosuppressed patients more often.(27) Additionally, these assays measure different aspects of T cell response. ELISpot/Fluorospot quantifies the number of T cells that respond whereas the interferon-y

release assay measures the amount of cytokine produced by T cells in response to antigen stimulation.

While the STAR SIGN study found decreased CMIR among those on anti-TNF therapy, the Impact of Biologic and Immunomodulatory Therapy on SARS-CoV-2 Infection and Immunity in Patients with Inflammatory Bowel Disease (CLARITY-IBD), Coronavirus Risk Associations and Longitudinal Evaluation-IBD (CORALE-IBD), and HumoRal and CellULar initial and Sustained immunogenicity in patients with IBD (HERCULES) study groups found that patients with IBD on anti-TNF therapy have similar or augmented CMIR following vaccination with the primary series. The CLARITY-IBD study examined humoral and CMIR to the BNT162b2 and ChAdOx1 nCOV-19 vaccines among patients with IBD treated with infliximab or vedolizumab.(28) Approximately one fifth of patients failed to mount a CMIR in both groups after the primary series. The CORALE-IBD study examined CMIR among 303 patients with IBD and found all participants had a T-cell clonal response. Spike-specific T cell response reduced significantly with age. There were no significant differences in response among those on ustekinumab, vedolizumab, tofacitinib, or steroids. CMIR was augmented among those on anti-TNF therapy after adjusting for age and sex. The HERCULES study found that the majority of patients with IBD developed a CMIR after receiving two doses of mRNA COVID-19 vaccines. Those on anti-TNF therapy had an augmented CMIR.(21) This finding was confirmed in the current study where CMIR was augmented in those on anti-TNF monotherapy at both time points after a third dose of COVID-19 vaccine. Those on anti-TNF combination therapy with an immunomodulator did not have an augmented response showing that an augmented CMIR is potentially linked to anti-TNF monotherapy alone. The reason for the augmented response on anti-TNF therapy is unknown, but is postulated due to the effects of TNF-α on vaccine induce humoral and cellular responses.(29) TNF alpha supports B cell maturation and down regulates T cell expansion. The lack of down regulation would result in an augmented CMIR and the blunted B cell maturation would cause lower antibodies in patients on anti-TNF therapy.(30, 31)

These findings would suggest that patient on anti-TNF monotherapy may be at lower risk for severe disease from SARS-CoV2 given the augmented CMIR since T cells can help prevent onset of severe disease for most cases.

The CMIR is responsible for immunologic memory, recognition of viral variants and clearance, generation, and maintenance of antibody responses, and is an important component of COVID-19 vaccine efficacy in addition to humoral immunity. The CMIR can protect against viral variants which may evade humoral immunity.(18) The VIP study, STAR SIGN study, and current study demonstrate discordant CMIR and humoral immune responses, suggesting that there is not a strong correlation between antibody and CMIR among patients with IBD.(21) Previous studies in healthy controls have also demonstrated discordance in CMIR and antibody response.(19) In contrast, The Impact of Biologic Therapy on SARS-CoV-2 Infection and Immunity IBD (CLARITY-IBD) study demonstrated a modest positive correlation between CMIR and antibody concentration among those receiving an mRNA vaccine.(28) The discordance in these results demonstrate that both humoral and cell mediated immunity must be evaluated to determine the impact of different immune modifying therapy on COVID-19 vaccine immunity. Each component only accounts for a part of vaccine-induced immunity.

Both mRNA-1273 (Moderna) and BNT162b2 (Pfizer-BioNTech) elicit a T helper cell type 1 immune response producing IFNy shown in other studies in which only minimal T helper cell type 2 response was measured. (32, 33) The presence of CMIR has been shown to protect individuals from infection, even in the absence of neutralizing antibodies.(34) SARS-CoV-2 specific cell-mediated immune responses appear to wane more slowly compared to antibody concentrations. Additionally, these CMIR recognize conserved viral antigens with less than 15% of T cells recognizing viral mutations sites. (35, 36) Therefore, CMIR could provide protection from viral variants. All patients with IBD in this study mounted CMIR which persisted over the vaccine dosing interval. Our study showed slower waning of CMIR compared to

antibody concentrations. Clinicians can consider that other benefits of CMIR, such as recognition of at least some viral variants, that have been demonstrated in other studies may offer some additional vaccine protection in patients with IBD.

Our study has several strengths. We evaluated sustained CMIR over multiple time points among patients on stable treatment regimens for at least two months. This allowed us to assess the effect of different immunosuppressive medications on vaccine response. We assessed CMIR six months after the third dose of vaccine and were able to include a small cohort evaluating immunogenicity after a fourth dose of vaccine. Our study is limited by the lack of a reference healthy control population, unable to evaluate the impact of disease activity on vaccine response and small representation of certain treatment regimens (tofacitinib, thiopurine monotherapy and systemic steroids). We were also limited in being unable to evaluate the real world effectiveness of vaccine in preventing breakthrough infections since the goal of HERCULES was evaluating humoral and cell mediated immunogenicity of COVID-19 vaccine. Patients in study were not followed or asked about breakthrough infections during the study period. Thus, we were unable to evaluate the correlation of breakthrough infections with CMIR or IBD therapy. Evaluating breakthrough infections is a complex issue since the goal of vaccination is preventing severe disease that results in hospitalization. However, some variants are highly contagious and result in higher rates of infections. Furthermore, the impact of IBD therapies on breakthrough infections has been variable in multiple studies. (37, 38) Future studies should evaluate CMIR and humoral immune response in a larger population of patients in different treatment arms.

In summary, we found that patients with IBD should receive a third dose of an mRNA vaccine to maximize the CMIR and this response is sustained for approximately six months. We also confirmed our previous finding that patients on anti-TNF therapy have an augmented CMIR

to mRNA COVID-19 vaccines.

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Table 2. Multivariable models evaluating cell-mediated immune response 28-65 days after a third dose of a COVID-19 vaccine.
N= 183

	Univariable			**Multivariable**		
	Beta-coefficient	95% CI	P-value	Beta-coefficient	95% CI	P
Age (continuous variable)	-51.16	-89.4; -12.9	**0.009**	-32.4	-70.4; 5.6	0.09
Male	1199.4	73.1; 2325.7	**0.037**	1152.4	77.2; 2227.5	**0.036**
mRNA vaccine type (mRNA-1273)	-25.2	-1165.1; 1114.8	0.965			
Type of Boosting (Heterologous) (n=14)	-348.2	-2940.4; 1794	0.749			
Previous COVID-19 Infection (n=16)	-1074.6	-3084.8; 935.6	0.293			
Medication use						
Aminosalicylate or no IBD therapy (n=29)	-1571.9	-3114.5; -29.4	**0.046**	-675	-2221.1; 869.5	0.389
thiopurine monotherapy (n=14)	-1879.5	-4004.2; 245.6	0.083			
anti-TNF monotherapy (n= 57)	2627.4	1459.3; 3795.3	**<0.001**	2056.9	826.3; 3287.5	**<0.001**
Anti-TNF combination therapy[a] (n=22)	939.2	-806.7; 2685.1	0.29			
Vedolizumab monotherapy (n= 33)	-1207.7	-2678.5; 263.1	0.107			
Ustekinumab monotherapy or combination[a] (n=23)	-1601.8	-3303.9; 100.2	0.065			
systemic corticosteroids (n=9)	-2805.9	-5407.4; -204.3	**0.035**	-1927.7	-4482.6; 639.2	0.14
Tofacitinib (n=5)	1284.9	-2203.8; 4773.7	0.468			

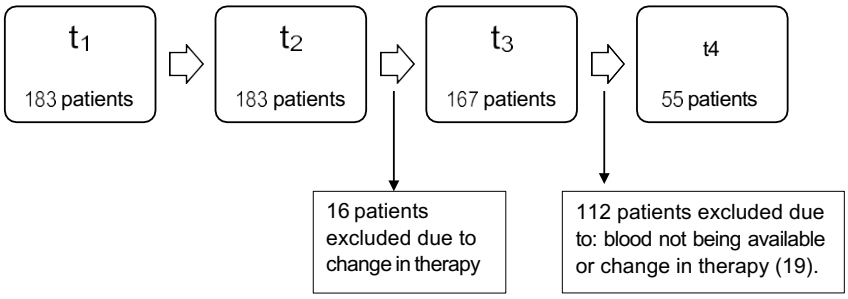
Abbreviations: TNF, tumor necrosis factor α.
[a] Including azathioprine, 6-mercaptopurine, or methotrexate.

Table 3. Multivariable models evaluating cell-mediated immune response approximately six months (+/- 45 days) after third mRNA dose (t_3) n=167						
	Univariable			**Multivariable**		
	Beta-coefficient	95% CI	P-value	Beta-coefficient	95% CI	P
Age (continuous variable)	-46.5	-85.6; -7.4	**0.0199**	-27	-66.3; 12.3	0.176
Male	783.4	-363.8; 1930.6	0.179			
mRNA vaccine type (mRNA-1273)	405.7	-746.4; 1557.9	0.488			
Type of Boosting (Heterologous) (n=12)	-188	-2421.1; 2044.3	0.868			
Previous COVID-19 Infection (n=38)	106	-1269.3; 1481.3	0.879			
Medication use						
Aminosalicylate or no IBD therapy (n=29)	-1725.8	-3224.7; -226.8	**0.0243**	-738.6	-2275.3; 789.1	0.344
thiopurine monotherapy (n=13)	-1308.9	-3451.8; 833.9	0.23			
anti-TNF monotherapy (n= 50)	2469.9	1269.3; 3670.3	**<0.001**	2089.3	815.7; 3362.8	**<0.001**
Anti-TNF combination therapy[a] (n=21)	652.8	-1083.5; 2389	0.459			
Vedolizumab monotherapy (n= 28)	-1350	-2879.6; 179.6	0.083			
Ustekinumab monotherapy or combination[a] (n=21)	-21.9	-1761.1; 1717.2	0.98			
systemic corticosteroids (n=12)	-404.6	-5347.3; 1392.6	0.721			
Tofacitinib (n=5)	-1977.4	-5347.3; 1392.6	0.248			

Abbreviations: TNF, tumor necrosis factor α.
[a] Including azathioprine, 6-mercaptopurine, or methotrexate

Figure 1

Flowchart of study participants in HERCULES study



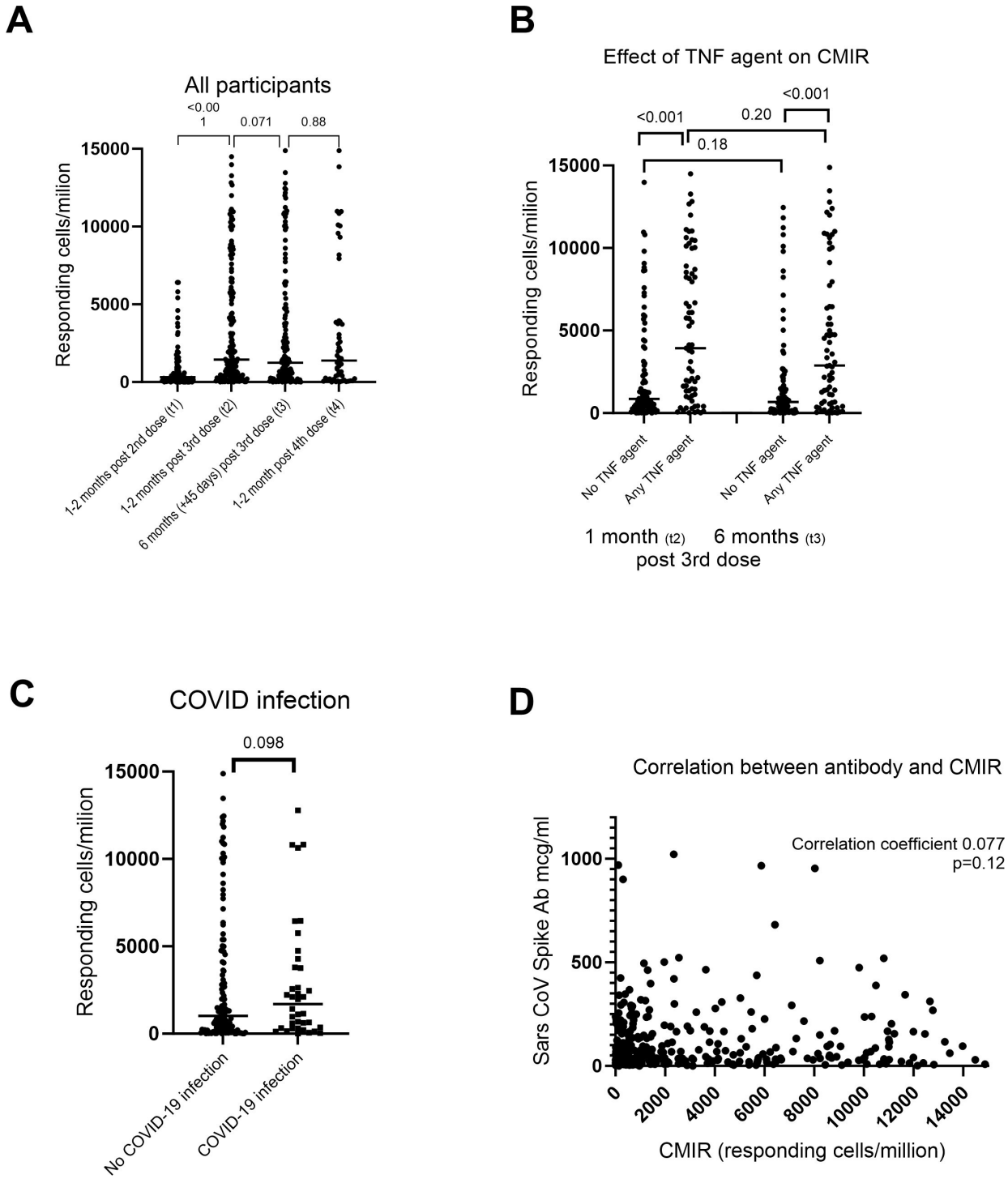


Figure legend.

Cell-mediated immune response (CMIR) following COVID-19 mRNA vaccines. A. CMIR increased after the 3rd vaccine dose (n=161; median 1444 responding cells/million (interquartile range (IQR) 421-6011) compared to after two vaccine doses (n=120; median 313 responding cells/million; IQR 94-960) p<0.001. The CMIR approximately six months after the 3rd dose was not statistically significantly different from 1 month after dose 3 (median 1241 responding cells/million (IQR 301-4609) Wilcoxon signed rank p=0.071). The CMIR did not boost following the 4th dose of vaccine (n=55; median 1387 responding cells/million (IQR 203-3843) p=0.88). B. Patients with IBD treated with TNF agents (n=69) had higher CMIR compared to those not treated with TNF agents (n=92) at both 28-65 days after 3rd dose (median 3927 responding cells/million (IQR 1094-8619) vs median 853 responding cells/million (IQR 343-2984) p<0.001) and at approximately six months post 3rd dose (median 2876 responding cells/million (IQR 614-7094) vs median 671 responding cells/million (IQR 161-2191) p<0.001). Additionally, neither group showed statistically significant waning of CMIR over 6 months. C. History of COVID-19 infection had no statistically significant effect on CMIR at 6 months after dose 3. (no COVID-19 infection median 1024 responding cells/million; IQR 228-4609 vs COVID-19 infection history median 1690 responding cells/million; IQR 311-3913; p=0.098). D. No statistically significant correlation was found between CMIR and SARS CoV spike antibody (Ab) concentrations (Spearman correlation coefficient 0.077; p=0.12). This graph shows data points from all time points post vaccines. CMIR-antibody correlations for each time point are included in the supplementary material.