T cell response after SARS-CoV-2 vaccination in immunocompromised patients with inflammatory bowel disease.

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Abstract

Background: Vaccination is a promising strategy to protect vulnerable groups like immunocompromised inflammatory bowel disease (IBD) patients from an infection with SARS-CoV-2. These patients may have lower immune responses. Little is known about the cellular and humoral immune response after a SARS-CoV-2 vaccination in IBD.

Methods: 28 patients with IBD and 27 age- and sex-matched healthy controls were recruited at Jena University Hospital. Blood samples were taken before, after the first and in a subgroup of 11 patients after second dose of a SARS-CoV-2 vaccination. Cellular immune response including IFN-γ and TNF-α response and antibody titers were analyzed.

Results: Overall, 71.4% of the IBD-patients and 85.2% of the controls showed levels of anti-SARS-CoV-2 antibodies above the cutoff of 33.8 BAU/ml (p=0.329) after the first dose. Even in the absence of SARS-CoV-2 antibodies, IBD patients showed significant T cell responses after first SARS-CoV-2 vaccination compared to healthy controls, which was not influenced by different immunosuppressive regimens. Associated with the vaccination, we could also detect a slight increase of the TNF production among SARS-CoV-2-reactive T_H cells in HD and IBD patients. After the second dose of vaccination, in IBD patients a further increase of humoral immune response in all but one patient was observed.

Conclusions: Already after the first dose of a SARS-CoV-2 vaccination, cellular immune response in IBD patients is comparable to controls, indicating a similar efficacy. However, close monitoring of long-term immunity in these patients should be considered.

Keywords: IBD**,** immunosuppression, vaccination, SARS-CoV-2, COVID19, Cellular immunity

Introduction

Since beginning of 2020, the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) pandemic has led to significant challenges in the treatment of patients with IBD. Although no increased susceptibility to SARS-CoV-2 infections or mortality is evident in patients with IBD, 17% of patients with IBD infected with SARS-CoV-2 had to be hospitalized worldwide[1] and patients with IBD express a great fear of getting infected[2]. The International Organization for the Study of Inflammatory Bowel Disease (IOIBD) and the COVID-19 European Crohn's and Colitis Organization (ECCO) taskforce recommended vaccinating all patients with IBD as soon as they are able to receive the vaccination, regardless of immune-modifying therapies[3,4]. COVID-19 vaccines are safe in patients with IBD[5] but they were excluded from COVID-19 vaccine trials and therefore efficacy is largely unknowen.[6]

A plethora of scientific reports describe impaired immunity after vaccination in IBD patients with immunosuppressive therapy. However, data regarding the effect of immunosuppressive therapies on the immune response is inconsistent. Upon vaccination against influenza[7–10] or pneumococci[11], IBD patients treated with immunosuppressive agents such as TNF-α-antibodies or receiving combination therapies developed lower humoral vaccine responses . In line with this, the immunogenity of hepatitis B vaccination was reduced in patients with IBD under treatment with immunosuppressive drugs.[12] Recently, studies in patients after solid organ transplantation found reduced antibody levels after the first [13,14] and the second dose of the vaccine.[15] In contrast to this, comparable vaccination responses of immunosuppressed and non-immunosuppressed individuals have been detected after vaccination against Influenza.[16,17] In patients with IBD and immunosuppressive therapy, the humoral immune response against SARS-CoV-2 was inadequate after first dose of vaccination[18]. Although in most of these studies the vaccination response was examined by detection of antibody titers, some concerns arose, whether it is adequate to only focus on humoral responses to estimate the potency and efficacy of different SARS-CoV-2 vaccines.

T-cell responses are as well crucial for immunity against SARS-CoV-2[19] and it is likely to develop after vaccination with SARS-CoV-2 vaccines. In the SARS-CoV T cells were associated with long-lasting immunity[20] while humoral response was only detectable for a short period.[21] The same kinetics of antibodies and T cells were described in SARS-CoV-2.[22] However, in IBD patients with immunosuppression, a T cell-response towards a SARS-CoV-2 vaccine has not been described, yet.[18] Therefore, it is necessary to analyze T cells responses in parallel to humoral immunity to evaluate a vaccination response in immunocompromised patients.

The primary objective of this study was therefore, to analyze the T cell response in parallel to the humoral immune response after one dose of a SARS-CoV-2 vaccine in patients with IBD under immunosuppressive therapies in comparison to untreated controls.

Methods

Patients with IBD treated in our outpatient clinic at Jena University Hospital were included in the study, if they had an appointment to get a SARS-CoV-2 vaccination. Blood samples were taken before the first dose of the vaccination and three weeks after. Patients' charts were reviewed to extract data on age, sex, type, manifestation and current activity of IBD and the IBD-related medication. Patients with previous PCR-proven SARS-CoV-2 infection or positive anti-SARS-CoV-2 antibodies before the first dose of the vaccine were excluded from analysis.

Health care workers without immunosuppression receiving the vaccination were recruited as controls and were matched for age and sex. The study was approved by the local ethics committee (2020-2045-BO) and written informed consent was obtained from all patients and controls before inclusion in the study.

Cellular measurements

Peripheral Blood Mononuclear Cells (PBMCs) were separated on a Biocoll solution (Bio&SELL GmbH, Germany) by centrifugation at 800 x g at room temperature (RT) for 20 min without brakes. Intermitting phase containing PBMCs was washed with PBS for 2 times and subsequently cryoconserved in liquid nitrogen in medium containing Penicillin/Streptomycin (Sigma-Aldrich), 10 % DMSO (Sigma-Aldrich) and 50 % FCS (Sigma-Aldrich). Upon thawing at RT, PBMCs were washed with cell culture medium (supplemented with 10% human AB serum (PAN Biotech, Germany), penicillin/streptomycin) and let rest at 37°C for 1h. Subsequently, a maximum number of 5 x 10^6 PBMCs were restimulated in cell culture medium containing 1 µg/mL recombinant anti-human CD28 antibody (clone CD28.2, BioLegend) with either 0.2 % DMSO (negative control), SARS-CoV-2 Spike glycoprotein PepMix 1 (S1, N-terminal coverage) or 2 (S2, C-terminal coverage) (both jpt, Germany). As high controls 10^6 PBMCs were restimulated with 1 µg/mL TSST1 and 1 µg/mL SEB (both Sigma-Aldrich) in presence of 1 µg/mL recombinant anti-human CD28 was used, or anti-human CD3/CD28 beads (Gibco/Thermo Fisher Scientific, Lithuania) at a ratio of 1 bead/PBMC. All samples were incubated for 2 h and Brefeldin A (BioLegend) was added for another 14h of incubation. Upon centrifugation at 300 x g at RT for 10 min, cells were recovered in 1 mg/mL beriglobin and stained with anti-human CD3 Pacific Blue (clone UCHT1, BioLegend) and anti-human CD4 Brilliant Violet 605 (clone RPA-T4, BioLegend). After 5 min, Zombie Aque fixable dead cells stain (BioLegend) was added and incubated for another 10 min. Upon washing with PBA/E, the cells were fixed in 2% Formaldehyde/PBS at RT for 20 min, intracellularly stained with anti-human CD154 APC (clone 24-31, BioLegend), anti-human CD137 PE/Cy7 (clone 4B4-1, BioLegend), anti-human IFNγ APC/Cy7 (clone 4S.B3, BioLegend), anti-human TNFα PerCP/Cy5.5 (clone MAb11, BioLegend), anti-human IL-4 PE (clone MP4-25D2, BioLegend), and anti-human IL-17A FITC (clone BL168, BioLegend) in 0.5%Saponine (Sigma-Aldrich) in PBA/E at 4°C for 20

min. Cells were recovered in PBA/E and analyzed with a FACS-Canto-Plus flow cytometer (BD). Data were analyzed with FlowJo V10.7 (BD, Ashland, OR, USA).

Serological measurements

Serological analyses for SARS-CoV-2 antibodies were performed using the Liaison SARS-CoV-2 Trimerics IgG CLIA on the LiaisonXL (DiaSorin, Saluggia, Italy) following the manufacturer's instructions. This assay detects IgG antibodies against SARS-CoV-2-specific trimeric spike glycoprotein with an estimated sensitivity of 98.7% (153/155) at ≥15 days after the first positive RT-PCR and an estimated specificity of 99.5% (1889/1899). Results are defined as seropositive for measured values of ≥13 AU/ml or ≥33.8 BAU/ml, respectively. According to the manufacturer, this assay has shown a positive agreement of 100% (Wilson 95% CI: 97.8-100%) when compared to a micro-neutralization assay, while the negative agreement is stated as 96.9% (Wilson 95% CI: 92.9-98.7%).

Statistical analysis

Statistical analysis was performed using SPSS v27 (IBM, Armonk, NY, USA) or Sigma Plot 13 (SYSTAT Software GmbH, Germany). Normal distribution was tested using the Shapiro Wilk test. If the test for normal distribution failed Mann-Whitney-U test was performed, otherwise significance was tested using a two-sided, non-paired Student's *t*-Test. Data are expressed as medians with interquartile range unless otherwise indicated. For categorial variables, the Fisher's exact test was used. P-values <0.05 were defined as significant.

Results

28 patients with IBD were included in the analysis, among them 17 patients with Crohn's disease (CD) and 10 patients with ulcerative colitis (UC) and one with not defined IBD. The median age was 42 years. Overall, nine patients had additional extraintestinal manifestations

and nine patients had previous IBD-related complications including surgery. IBD was in remission in 20 patients and chronic-active in eight patients. All patients received immunosuppressive medication at inclusion. Two patients with UC had additional liver transplantation due to primary sclerosing cholangitis and one patient received a heart transplantation due to non-IBD associated disease. Details on the baseline characteristics are presented in **Table 1**. Additionally, 27 healthy volunteers (HD = healthy donors) were included as a control group and were matched for age and sex. Both groups received either the AstraZeneca vaccine (ChAdOx-1) in 18 patients and 14 controls and an mRNA-based vaccine in 10 patients and 13 controls (BioNTech/Pfizer, BNT162b2) in the first dose, all received an mRNA vaccine as the second dose.

Assessment of cellular immune response

To quantify SARS-CoV2-specific T_H cells among $CD45^+$ PBMCs, we incubated the PBMCs with two S-Protein-derived peptide mixes covering the whole sequence of the Spike protein (N- and C-terminally, S-Mix1 or S-Mix2 respectively). When we analyzed the $CD137^+CD154^+$ (antigen-specific) cells among living $CD4^+CD3^+$ cells (T_H cells, **gating strategy is shown in Figure 1 A**) upon SARS-CoV-2 vaccination, we observed a comparable significant increase in frequencies of S-Protein-specific T_H cells in HD as well as in IBD patients **(Figure 1B, C)**. Despite we detected slightly increased S-Mix2-specific T_H cells in naïve patients as described by Braun et al.[23], this was not significant in both cohorts **(Figure 1C)**. However, we observed the presence of S-Mix2-reactive T_H cells prior vaccination at the level of IFNγ-producing T_H cells in HD and IBD patients **(Figure 1D)**. Of note, the SARS-CoV-2 vaccination resulted in an increase in the frequencies of IFN-γ producers among SARS-CoV2-reactive T_H cells in the HD as well as in the IBD cohort **(Figure 1D)**. Associated with the vaccination, we could also detect a slight increase of the TNF production among SARS-CoV-2-reactive T_H cells in HD and IBD patients **(Figure 1 E)**, but not of the IL-17A or IL-4 production (data not shown). Such vaccine-related immunogenic effects were similar between HD and IBD cohorts when separated by their deficiency of generating a sufficient

SARS-CoV2-specific antibody response **(Supplement-Figure 1A)**. IBD patients, who received an organ transplant did as well generate detectable SARS-CoV-2-specific T_H cell responses upon vaccination **(Supplemental Figure 1B)**.

A subcohort of the vaccinated subjects was reanalyzed after a second round of vaccination, in this group, the observed vaccine-related induction of SARS-CoV2-reactive T_H cells did remain in IBD patients **(Figure 1 F)** and thereby, this demonstrated that treated IBD patients did indeed develop a cellular SARS-CoV-2 specific immunity upon vaccination. In line with the results obtained from IBD patients after the first round of vaccination, these detected SARS-CoV-2 specific T_H cells contained non-significantly increased IFNγ producers **(Figure 1 G)** and showed a pronounced increase of TNFα-producing T_H cells **(Figure 1 H)**.

In a general analysis of $CD4^-$ T cells, which are predominantly $CD8^+$ cytotoxic T cells (T_C cells), we did not detect a deficiency of very faint overall CD137 upregulation in IBD patients upon vaccination **(Supplement-Figure 1 C)** or of the faintly induced IFNγ production among them **(Supplement-Figure 1 D)**. Of note, a more detailed analysis of $CD137^+$ Tc cells is necessary to study significant immunogenic effects of a SARS-CoV2 vaccine in IBD patients on the T_C cell population, including detection of CD69 or specifity via pMHC multimers.[24]

Assessment of humoral immune response

Overall, in both groups, one person showed positive SARS-CoV-2 IgG antibodies indicating an inapparent infection before vaccination, these patients were excluded from further analysis. Three weeks after the first dose of the vaccine, 20 of the IBD patients (71.4%) had detectable levels of SARS-CoV-2 antibodies, indicating an immunological response to the vaccine, while 8 patients (28.6%) showed levels below the cutoff of 33.8 BAU/ml. Interestingly, there was a sufficient antibody production in 23 of the healthy controls (85.1%) as well and it was still below the cutoff in four healthy controls (14.9%). The difference between both groups was not statistically significant **(Figure 2A**, p=0.329**)**. Furthermore, when looking at the level of SARS-CoV-2 antibodies, they were slightly, but non-significantly

higher in the healthy controls **(Figure 2B,** median 57.2 vs 105.0 BAU/ml, p=0.113**)**. 12 patients and 12 controls already received the second dose of the vaccination. In these patients, antibodies markedly increased and were detectable in all samples of the healthy donors and all but one patient (91.7%). Still, the antibody titers were slightly higher in the healthy donors. (1119 vs. 1570 BAU/ml, p=0.313). 4 of the patients analyzed after the second vaccine dose did not have detectable antibodies after the first dose, interestingly, 3 of these patients developed positive titers after the second dose, however, the levels were lower than in patients with already positive titers after the second dose and comparable to the levels found after the first dose in the already positive patients (Figure 2A)

To determine the impact of different types of immunosuppression, we stratified the patients according to their therapy. Unexpectedly, we could not observe any difference between anti-SARS-CoV-2 antibodies in patients with and without TNF-antibodies (**Figure 3,** p=0.629), with ustekinumab therapy (**Figure 3,** p=0.371). Additionally, the number of systemic immunosuppressive drugs taken by a patient did not have an impact on the antibody levels post vaccination (**Figure 3,** Spearman's rho = -0.216, p=0.270). Patients taking only one immunosuppressive drug had comparable levels of SARS-CoV-2 antibodies than patients taking more than one drug (**Figure 3,** p=0.566).

We have included 3 patients with additional solid organ transplantation (2 LTX and 1 HTX). As these patients have a more complex immunosuppressive therapy, we performed a separate analysis comparing the transplant and non-transplant IBD-patients. The antibody response in these patients showed a huge variety between the lower limit of detection in the patients after HTX and one of the LTX patients, up to 1550 BAU/ml in the other LTX patient. The IBD patients without concomitant solid organ transplantation had a median level of 57.5 BAU/ml. (**Figure 3**) Notably, the patients without antibodies had a significant increase in SARS-CoV-2 specific T_H cells comparable to patients without transplantation, indicating an effect of the vaccination

Discussion

In this study, we demonstrate a significant SARS-CoV-2 specific cellular immune response in 27 immunocompromised patients with IBD after one dose of a SARS-CoV-2 vaccine. The T-cell response was similar to that in healthy controls, indicating a protective effect of the vaccine in immunocompromised patients with IBD. The humoral response was sufficient in only 71.4% of the patients following the first dose and 91.7% after the second dose.

Recent studies raised concerns about the efficacy of the SARS-CoV-2 vaccination in immunosuppressed patients. Antibody levels after vaccination were found to be up to 20% lower compared to healthy controls after a single dose of a vaccine in transplant patients[14]. In line with this, kidney transplant recipients showed positive SARS-CoV-2 antibodies after vaccination in only 5-10%[13,15] and presented a weak T-cell response measured by ELISPOT assay as well[15]. A recent study in patients vaccinated with the BioNTech/Pfizer vaccine after liver transplantation (LTX) found antibodies in 47.5% of the patients and 100% of the controls.[25] The authors identified co-medication with mycophenolate or steroids as a risk factor for vaccination failure, both were used only in the transplant patients in our cohort. Additionally, higher age was associated with an increased risk of vaccination failure[25]. Another recent study found adequate antibody response in 86% of rheumatologic patients following a SARS-CoV-2 vaccination, which is in line with our findings.[26]. The authors could identify treatment with rituximab as a risk factor for non-response to the vaccine, which was not used in our cohort. We did include two patients with IBD and LTX due to PSC and one patient with HTX. Of these three patients, one patient developed antibodies. However, our patients with IBD were younger than the patients in the transplant cohorts. A recent study on transplant patients found positive antibodies in 40% of patients after the second dose and the authors were able to increase these proportion to 68% using a third dose of an mRNA SARS-CoV-2 vaccine.[27] Data on the immune response in patients with IBD after vaccination are sparse. A recent study reported adequate humoral immune responses after the second dose or after one dose and prior infection, which is in line with our findings, but antibody

titers in this study were lower in patients treated with Infliximab compared to vedolizumab.[18] Additionally, the same group reported the same differences in antibody titers in patients with IBD after confirmed SARS-CoV-2 infection[28]. However, data on cellular immune response after SARS-CoV-2 vaccinations have thus far not been reported in patients with IBD. Thieme et al. have investigated SARS-CoV-2-specific immune responses in another population of immunosuppressed patients, i.e., transplant patients.[29] Interestingly and in accordance with our data, they found no differences in humoral or cellular immune response between the transplant patients and controls.

In patients with negative antibody titers after the first vaccination, 3 out of 4 patients had detectable levels after the second dose, which were on the same level als titers after first vaccination in controls and IBD patients, who had yet positive titers after the first dose. It is tempting to speculate, that these patients might have a benefit of another booster vaccination dose as recently shown in transplant patients[27], but as the number of patients is small, a larger cohort of patients is needed

Most importantly, we were able to show an increase in SARS-CoV-2-specific T_H cells in IBD patients already after the first SARS-CoV-2 vaccination. We found that these SARS-CoV-2-specific T_H cells were maintained or even enhanced upon a second dose of vaccine. While in non-immunized, non-infected donors such SARS-CoV-2-reactive T_H cells have been described by Braun et al., who termed such subjects reactive healthy donors.[23] However, little is known about SARS-CoV-2 specific T-cells in patients with IBD.[6] In line with cross-reactive antigen-specific T-cells being present in up to 35% of healthy donors in other studies[23], we detected the presence of such reactive healthy donors in our HD as well as IBD cohorts prior vaccination. The protective or pathogenic relevance of such pre-existing SARS-CoV-2 specific T_H cells is currently a matter of debate.[30–32] They might represent either memory cells from a former encounter with SARS-CoV-2 (as in the additionally antibody positive patient), or are cross-reactive T_H cells originating from other infections, e.g. previous infections with common coronaviruses.[23] Collectively, we could show that IBD

patients independent from their medical history do partially possess cross-reactive SARS-CoV-2-specific T_H cells comparable to healthy donors, and that vaccination of IBD patients did induce a robust T_H cell-mediated immune response against the viral spike protein.

Two of the IBD patients showed low levels of TNF- and IFN- producing $CD137^+/CD154^+CD4^+$ T cells following the vaccination, indicating a potential weaker response. However, low frequency of positive cells has to be taken in to account in interpreting these results. Nevertheless, both of these patients had positive anti-SARS-CoV-2 antibodies following vaccination.

Our study has some limitations. First, we mainly examined the response following the vaccination after the first dose, and only in a smaller subcohort after the second dose. However, for all vaccines used in the participants, a second dose is strongly recommended. In line with other studies, who detected a sufficient T-cell response after one dose of a SARS-CoV-2 vaccine[33,34], we observed a robust increase of SARS-CoV-2-specific T_H cells in immunosuppressed IBD patients, which was preserved throughout a second vaccination. Second, we included both, AstraZeneca and BioNTech/Pfizer vaccines. In the current discussion about SARS-CoV-2 vaccinations, the recommendations regarding the AstraZeneca vaccine, which was used in the majority of the patients as the first dose, changed several times. As our patients were younger than 60 years, they will get another type of vaccine as the second dose, following current German recommendations, which is the BioNTech/Pfizer vaccine in most cases. We therefore decided to include both types of vaccine in the current study. Third, data on the duration of the immune response is lacking and we cannot exclude a shorter duration of immunity following vaccination in immunosuppressed IBD patients. While we still detected SARS-CoV-2-specific Th cells with an increased tendency of IFNγ production upon the second vaccination, a monitoring of a robust long term immune response is lacking. Forth, the sample size is still small and larger cohort studies are needed to validate the observed vaccination-induced immune protection of immunosuppressed IBD patients from SARS-CoV-2.

Nevertheless, our data indicates an adequate humoral and cellular immune response in immunosuppressed patients with IBD, indicating a comparable efficacy to healthy controls. Therefore, monitoring of the vaccination effect and long-term immunity should be considered.

Authors' contribution

PAR, and NA performed statistical analyses and wrote the manuscript. NA and SG performed experiments. PAR and AS conceived the study. PAR, PG and AS provided patient samples. AS and TK gave important intellectual input and interpreted the data. All authors critically revised the manuscript for important intellectual content. The authors thank Prof. A. Scherag for help with statistical analyses.

Data Availability Statement

The data underlying this article is available on reasonable request from the corresponding author

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Figure legends

Figure 1: PBMCs from 16 healthy donors (healthy) or 23 patients with inflammatory bowel disease (IBD) were analyzed for S-Protein-specific T_H cells. DMSO (solvent of S-peptide mixes) was used as control. S-protein mixes 1 (S-Mix1) and 2 (S-Mix2) represent the S-Protein N-terminal part and C-terminal part, respectively. **A,B)** Gating strategy is shown in (A) and S-Protein-specific T_H cells are depicted as $CD137^+CD154^+$ among living $CD4^+CD3^+$ in (B). **C)** Data of all 16 healthy donors and 23 IBD patients are summarized in the box plots. **D,E)** IFNγ producing cells (D) or TNFα-producing cells (E) among $CD137^+CD154^+$ T_H cells are summarized in box plots. F-H) Upon a second round of vaccination, PBMCs from subjects of the IBD group were restimulated and analyzed as in (A-E). Statistics were analyzed as described in material and methods section. *p<0.05, **p<0.01, ***p<0.001, n.s.non-significant.

Figure 2: Levels of SARS-CoV-2 antibodies in patients and controls before and after the first and second dose of a SARS-CoV-2 vaccination.

Figure 3: Titers of SARS-CoV-2-specific antibodies were stratified according to their immunosuppressive treatment. Statistics were performed as indicated in Material and Methods section. $^{n.s.}$non-significant

Tables

	IBD-patients
Sex (Male/Female)	15/13
Age	42 (36; 59)
Active smoker	3 (10.7%)
Diagnosis (CD/UC/other)	17/10/1
Current activity Remission Chronic-active disease	 20 (71.4%) 8 (28.6%)
Extra-intestinal manifestations	8 (28.6%)
Previous IBD-associated complications Fistula Stoma Abscess Pouch Stenosis	9 (32.1%) 6 (21.4%) 2 (7.1%) 3 (10.7%) 2 (7.1%) 1 (3.5%)
Previous transplant	3 (10.7%)
Current immunosuppressive medication	
Any immunosuppression	28 (100%)
Steroids	2 (7.1%)
TNF-Antibodies	9 (32.1%)
Vedolizumab	3 (10.7%)
Ustekinumab	8 (28.6%)
Azathioprin	3 (10.7%)
Mycophenolate	2 (7.1%)
Tacrolimus	3 (10.7%)
Tofacitinib	1 (3.5%)

Table 1: Baseline characteristics of the IBD-patients. Data are presented as absolute number and percentage or as median and inter-quartile range

Figure 1

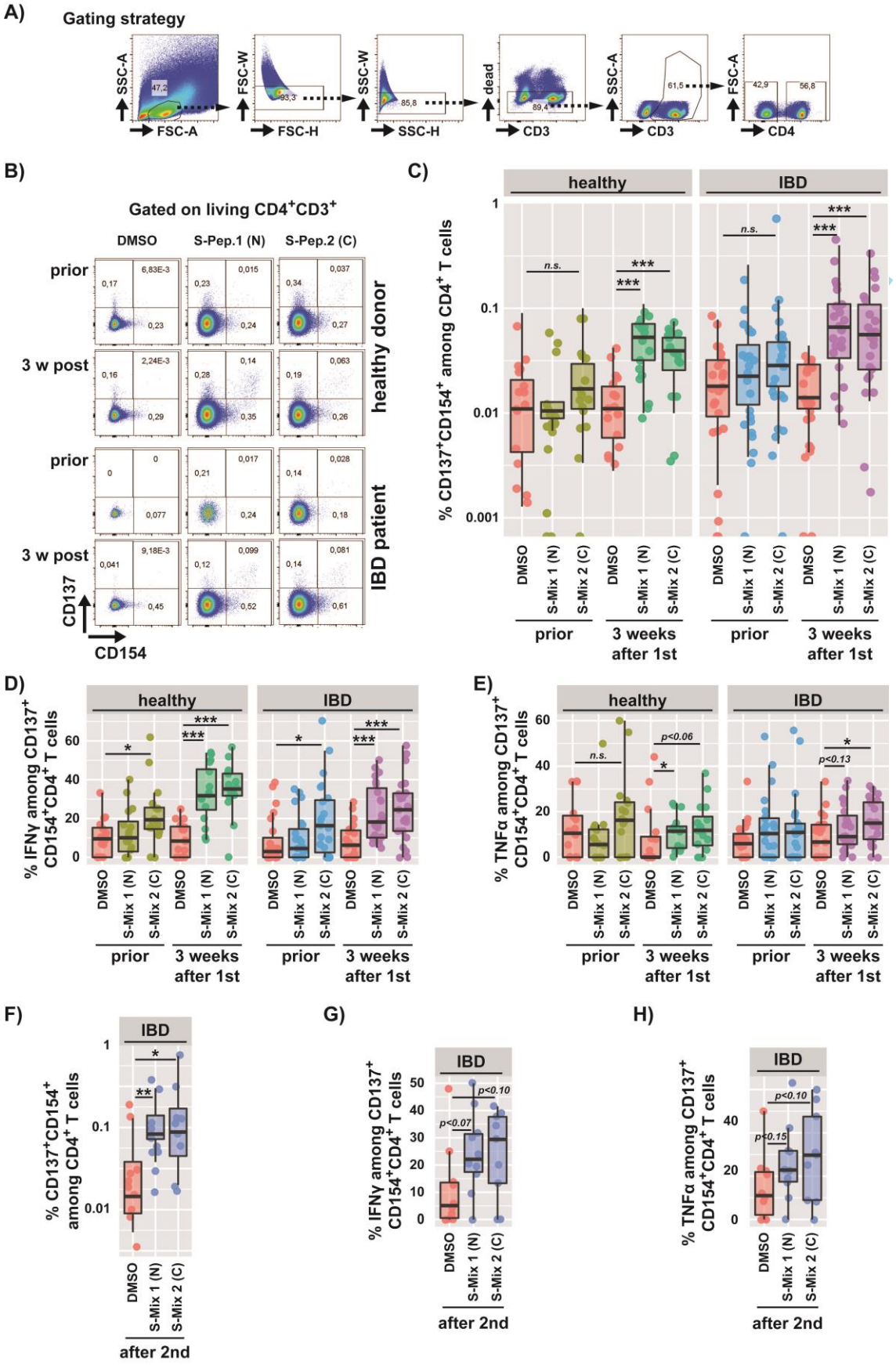
A)
Gating strategy
SSC-A
FSC-A
FSC-W
FSC-H
SSC-W
SSC-H
dead
CD3
CD4
B)
Gated on living CD4+CD3+
DMSO
S-Pep.1 (N)
S-Pep.2 (C)
prior
3 w post
healthy donor
IBD patient
CD137
CD154
C)
healthy
IBD
% CD137+CD154+ among CD4+ T cells
DMSO
S-Mix 1 (N)
S-Mix 2 (C)
prior
3 weeks after 1st
D)
% IFNγ among CD137+ CD154+CD4+ T cells
E)
% TNFα among CD137+ CD154+CD4+ T cells
F)
% CD137+CD154+ among CD4+ T cells
after 2nd
G)
% IFNγ among CD137+ CD154+CD4+ T cells
H)
% TNFα among CD137+ CD154+CD4+ T cells

Figure 2

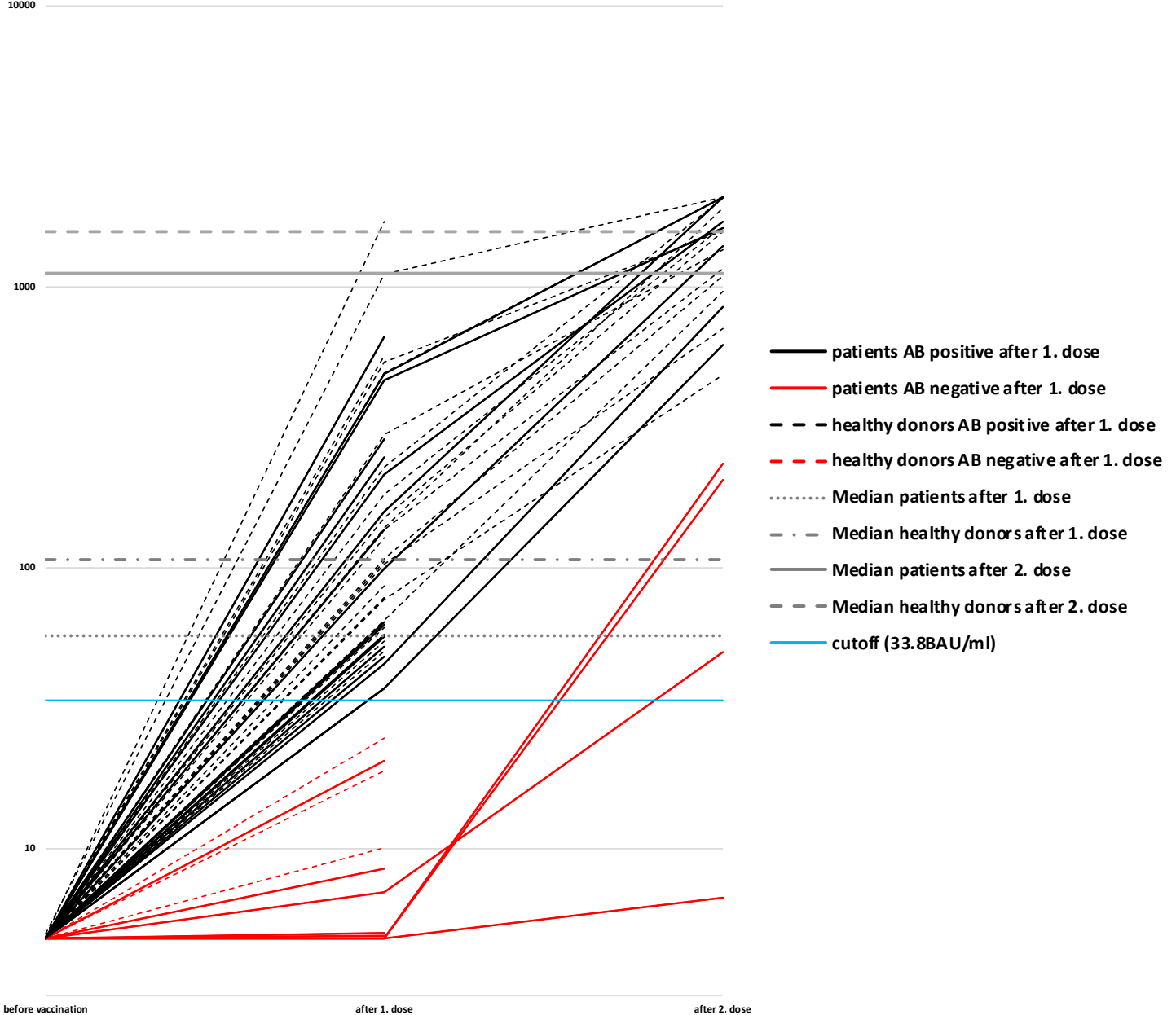
10000
1000
100
10
before vaccination
after 1. dose
after 2. dose
patients AB positive after 1. dose
patients AB negative after 1. dose
healthy donors AB positive after 1. dose
healthy donors AB negative after 1. dose
Median patients after 1. dose
Median healthy donors after 1. dose
Median patients after 2. dose
Median healthy donors after 2. dose
cutoff (33.8BAU/ml)

Figure 3