

Significant Production of Serum and Mucosal Anti-Spike-IgA Antibodies After Vaccine-Encoded or SARS-CoV-2-Infection-Induced Spike-Exposures in Patients with Asthma Treated with Monoclonal Antibodies Compared to Conventional Therapy

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Introduction: It has been proposed that patients with asthma on monoclonal antibodies (mAb) targeting Interleukin-5 (IL-5), IL-4/IL-13 pathways or IgE may demonstrate insufficient defense against viral infections requiring strong T-helper-cell-type-1-(Th1) for neutralizing antibody production and cytotoxic CD8+ T-cell responses for efficient clearance of the viral pathogens. It is a matter of debate whether those mAb may impair the immune response against SARS-CoV-2-spike-protein by interacting with cytokines critical for B-cell differentiation and antibody maturation. This controlled cross-sectional cohort study aimed to characterize the mucosal and serum humoral immune response as well as the cellular reactivity against spike-protein in asthma patients on mAb or on conventional treatment (conv).

Materials and Methods: Nasal and serum anti-spike-IgG and -IgA concentrations, avidity, neutralizing IgG and cytokine profiles were assessed using serological and neutralization assays and bead-based cytokine detection in nine patients on mAb matched to nine patients on conv who had received COVID-19-mRNA-vaccination. Proportions of spike-induced subpopulations of T- and B-cells were investigated by flow cytometry.

Results: Blockade of IL-5 and IL-5 receptor showed higher serum and nasal concentrations of anti-spike-IgA against recombinant-binding-domain-(RBD) and spike-protein-1-(S1) and similar concentrations of anti-spike-IgG compared to mAb or conv therapy. A spike-specific CD8+ T-cell-driven immune response with increased cytotoxic markers was seen in anti-IL-5 treatments. Baseline Th1 responses correlated with IFN γ - and TNF α -production in supernatants of spike-protein-stimulated cultures in all patients.

Conclusion: The findings indicate a significant specific adaptive immune response to SARS-CoV-2-spike-protein exposures by Th1- and Th2-driven responses with a significant response by serum and mucosal anti-S1 and anti-RBD-IgA in anti-IL-5-treated patients compared to IL-4/IL-13-targeting, anti-IgE or conventional therapies. Thus, based on the results it may be expected that immunogenicity of COVID-19-mRNA-vaccines or infection-induced spike-exposures is equivalent between asthma patients on monoclonal antibodies compared to those treated with conventional therapy.

Keywords: asthma, conventional therapy, IL-5, monoclonal antibody therapy, SARS-CoV-2



Introduction

Over the past few years, an increasing number of monoclonal antibodies (mAb) have been approved for use as add-on therapy for severe asthma. These antibodies are targeting Th2-driven factors such as interleukin-5-(IL-5)/IL-5-receptor- α -(IL-5R), and can also affect IL-4 and IL-13, as well as antibody secretion of the immunoglobulin E (IgE) type from activated leucocytes. Mepolizumab directly inhibits IL-5, while benralizumab blocks the IL-5R which drastically reduces eosinophilic activation.¹ The IL-5R is also found on B cells, where an IL-5 stimulus increases the production of IgA.² Omalizumab acts as an anti-IgE-antibody leading to less activation of mast cells and eosinophiles.³ Dupilumab inhibits IL-4/IL-13, resulting in less Th2 activation and reduced IgE production in B cells.⁴ These mAbs reduce the use of glucocorticoids and diminish inflammatory exacerbations while enhancing lung-function.^{5,6} These therapeutic options may affect innate immune cell or adaptive immune cell phenotypes against infections where a preferential T helper cell type I (Th1) response is generally considered as facilitating neutralizing antibody (nAb) production. However, as these antibodies also modulate the adaptive antibody response which requires Th2 function for refinement of antibodies,⁷ they possibly influence the immune reaction against certain infections.^{8,9} Particularly the class-switch towards mucosal antibodies of IgA type as well as affinity maturation of IgG antibodies requires functional active IL-5 and IL-4^{10,11} derived from Th2 cells. IgA is distinguished into IgA1 and IgA2, with IgA1 being more prevalent in serum. The IgA dimeric form mainly constitutes the secretory IgA and provides higher binding capacity than the monomeric form.¹² It has been emphasized that IgA plays a crucial role in anti-viral defense of SARS-CoV-2.¹³

People with pulmonary diseases have a higher risk of developing severe sequelae of COVID-19 compared to the immunologically healthy population.¹⁴ In those patients, particularly lower respiratory tract infections may be complicated by leukocyte interactions including mast cells and macrophages with type I and type II pneumocytes in conjunction with cytokines and chemokines. While an elevated risk has been discussed for asthma patients at the beginning of the pandemic,^{15,16} clinical evidence suggests that asthma patients on low doses of inhaled glucocorticoids do not represent a risk group for severe omicron infection.^{17,18} However, the effect of mAb therapy on establishing efficient humoral and cellular immune responses against severe COVID-19 manifestations is still unclear. For prevention of severe COVID-19 morbidity caused by SARS-CoV-2, COVID-19-mRNA vaccinations have been shown to be effective to protect against severe COVID-19 in the case of basic immunity mediated by three antigen exposures,¹⁹ either by vaccine-derived or infection-induced spike-protein, as been recommended for all adults.^{20,21} Recently, lower post-vaccination immunity towards SARS-CoV-2 after two doses of SARS-CoV-2 mRNA vaccination was shown in a study investigating patients with asthma or atopic dermatitis taking benralizumab, dupilumab or mepolizumab.⁸ In the era whereas SARS-CoV-2 infections tend to endemic state and yearly vaccinations against COVID-19 with adapted mRNA vaccines are subject to recommendation for elderly and immunocompromised individuals, the anti-viral response to other respiratory pathogens or to mRNA vaccines encoding for the prefusion-protein of respiratory syncytial virus (RSV) become of interest.²²

Although a review highlighted the overall morbidity reduction post-vaccination in terms of antibody production,^{23,24} to our knowledge, there is still a lack of information regarding the SARS-CoV-2-spike-specific cellular and humoral immune responses to SARS-CoV-2 after primary COVID-19-mRNA vaccination with two doses and a first or second booster dose with either omicron BA.5-adapted or non-adapted in asthma patients on mAb or on conventional treatments (conv). Therefore, this cross-sectional controlled pilot-study aimed to evaluate the concentrations of anti-spike-IgG and class-switch towards serum and mucosal IgA antibodies, avidity maturation and neutralization capacity of anti-spike-IgG as well as to characterize the T and B cell immune response to spike-protein in asthma patients on mAb compared to conv.

Materials and Methods

Study Design and Data Collection

Nine patients diagnosed with asthma on mAb were randomly 1:1 matched regarding age to nine asthma patients on conv and consecutively enrolled in this cross-sectional, single-center pilot study at the Department of Pneumology and Critical Care Medicine, University of Heidelberg, excluding patients with immunosuppressive drugs or high dose oral glucocorticoids (OCS) >10 mg/d, monogenetic immunodeficiency syndromes, acquired immunodeficiency, history of malignancy, severe infection requiring provision of healthcare, including doctor consultations, diagnoses and treatment or hospitalization in the last 4 weeks, blood products or other vaccinations in the last three months (Table 1 and Table 2).

Table 1 Demographics of Study Group

	Patients on mAb	Patients on conv	p-value
Number	9	9	–
Sex (female) (n; %)	4; 44.4	4; 44.4	1.000
Age (years)	62 ± 10 (62; 51–83)	53 ± 16 (57; 25–76)	0.251
Medication			
Oral glucocorticoids OCS (n; %)	1; 11.1	4; 44.4	0.158
Inhaled glucocorticoids ICS (n; %)	9; 100.0	9; 100.0	1.000
LAMA (n; %)	5; 55.5	9; 100.0	0.082
LABA (n; %)	9; 100.0	9; 100.0	1.000
(n; %) Mepolizumab	2; 22.2	0; 0	0.001
(n; %) Benralizumab	2; 22.2	0; 0	0.001
(n; %) Dupilumab	2; 22.2	0; 0	0.001
(n; %) Omalizumab	3; 33.3	0; 0	0.001
Clinical data			
Eosinophiles (n/μL)	484 ± 726 (215; 0–2170)	104 ± 118 (70; 0–390)	0.135
IgE (U/mL)	302 ± 209 (202; 4–852)	1588 ± 2435 (215; 1–7444)	0.441
FeNO (ppb)	26 ± 16 (18; 0–49)	42 ± 35 (34; 0–125)	1.000
Asthma endotype (mixed/allergic/intrinsic) (n/n/n)	9/0/0	5/2/2	0.076
Smoker (ex/never) (n/n)	7/2	5/4	0.620
BMI (kg/m ²)	30.5 ± 4.4 (30.9; 23.7–39.1)	30.6 ± 5.6 (29.8; 22.5–39.3)	0.965
FEV1 (%)	75.4 ± 15.3 (78.0; 53–99)	57.7 ± 23.6 (50.0; 35–99)	0.347
FEV1/FVC (%)	71.4 ± 11.9 (74.4; 50.4–87.0)	65.2 ± 20.9 (56.3; 50.0–100)	0.347
RV/TLC (%)	41.8 ± 9.2 (45.9; 27.9–56.9)	47.5 ± 13.1 (50.6; 21.4–63.2)	0.347
Influenza vaccination in the last 2 years (yes; %)	4; 44.4	7; 77.8	0.335
Pneumococcal vaccination in the last 2 years (yes; %)	3; 33.3	3; 33.3	1.000
Infection and vaccination data			
One vaccination (n; %)	0; 0	1; 11.1	0.584
Three vaccinations (n; %)	7; 77.7	6; 66.6	0.584
Four vaccinations (n; %)	2; 22.2	2; 22.2	0.481
Vaccination on biologics (n; %)	9; 100.0	0; 0	0.001
Omicron-adapted vaccine	2; 22.2	2; 22.2	1.000
SARS-CoV-2 infection (n; %)	5; 55.5	5; 55.5	1.000
Time since last exposure (months)	13 ± 4 (15.0; 2.0–17.0)	15 ± 18 (14; 12.0–18.0)	0.473

Notes: Clinical data are provided at the time point of blood withdrawal. Inhaled glucocorticoids were given at a maximum of 180 mcg twice a day. Oral glucocorticoids were administered between 20 to 30 mg per day. If not indicated otherwise, all continuous variables are given in mean ± standard deviation (median; minimum – maximum). Independent continuous non-parametric variables were compared by Mann–Whitney–U test, dichotomous variables by Fishers exact test.

Abbreviations: LABA, rapid-onset long-acting β_2 -agonist; LAMA, long-acting muscarinic antagonist; IL, interleukin; FeNO, forced expiratory nitric oxide; BMI, body mass index; FEV1, forced expiratory volume 1 second; FVC, forced vital capacity; RV, residual volume; TLC, total lung capacity; d, day.

Table 2 Demographics of mAb Group Stratified Into Anti-IL-5/Anti-IL-5R Therapy and Other mAb Therapy

	Patients on Anti-IL-5/Anti-IL-5R	Patients on Other mAb	p-value
Number	4	5	
Sex (female) (n; %)	0; 0	4; 80.0	0.056
Age (years)	58 ± 7 (57.0; 52.0–69.0)	65 ± 11 (6; 5–83.0)	0.462
Medication			
Oral glucocorticoids OCS (n; %)	0; 0	1; 20	0.329
Inhaled glucocorticoids ICS (n; %)	4; 100.0	5; 100.0	1.000
LAMA (n; %)	2; 50.0	3; 60.0	0.072
LABA (n; %)	4; 100.0	5; 100.0	1.000
Mepolizumab (n; %)	2; 50.0	0; 0	0.01
Benralizumab (n; %)	2; 50.0	0; 0	0.01
Dupilumab (n; %)	0; 0	2; 40.0	0.01
Omalizumab (n; %)	0; 0	3; 60.0	0.01
Clinical data			
Eosinophiles (n/μL)	47 ± 45(50; 0–90)	746 ± 832 (330; 180–2170)	0.025
IgE (U/mL)	350 ± 435 (114; 83–852)	273 ± 265 (290; 4–616)	0.655
FeNO (ppb)	40 ± 0 (40; 0–40)	22 ± 16 (16; 0–49)	0.400
Asthma endotype (mixed/allergic/intrinsic) (n/n/n)	4/0/0	5/0/0	0.273
Smoker (ex/never) (n/n)	4/0	3/2	0.273
BMI (kg/m ²)	30.2 ± 2.7 (31.1; 26.3–32.4)	30.7 ± 5.7 (30.9; 23.7–39.1)	0.806
FEV1 (%)	81.5 ± 15.3 (85.0; 84–99)	70.6 ± 13.5 (74.0; 53–90)	0.206
FEV1/FVC (%)	78.4 ± 9.5 (81.9; 62.5–87.0)	65.7 ± 10.7 (64.1; 50.4–80.6)	0.206
RV/TLC (%)	35.1 ± 8.2 (31.7; 27.9–49.1)	47.2 ± 5.7 (46.8; 39.2–56.9)	0.524
Influenza vaccination in the last 2 years (yes; %)	2; 50.0	2; 40.0	0.333
Pneumococcal vaccination in the last 2 years (yes; %)	0; 0	3; 60.0	0.165
Infection and vaccination data			
One vaccination (n; %)	0; 0	0; 0	1.000
Three vaccinations (n; %)	3; 75.0	4; 80.0	0.827
Four vaccinations (n; %)	1; 25.0	1; 20.0	0.893
Vaccination on biologics (n; %)	4; 100.0	5; 100.0	1.000
Omicron-adapted vaccine	1; 25.0	1; 20.0	0.984
SARS-CoV-2 infection (n; %)	3; 75.0	2; 40.0	0.567
Time since last exposure (months)	11.0 ± 6.2 (13.5; 2.0–15.0)	14.8 ± 2.2 (16.0; 12.0–17.0)	0.172

Notes: Clinical data are provided at the time point of blood withdrawal. Inhaled glucocorticoids were given at a maximum of 180 mcg twice a day. Oral glucocorticoids were administered between 20 to 30 mg per day. If not indicated otherwise, all continuous variables are given in mean ± standard deviation (median; minimum – maximum). Independent continuous non-parametric variables were compared by Mann–Whitney-U test, dichotomous variables by Fishers exact test.

Abbreviations: LABA, rapid-onset long-acting β_2 -agonist; LAMA, long-acting muscarinic antagonist; IL, interleukin; FeNO, forced expiratory nitric oxide; BMI, body mass index; FEV1, forced expiratory volume 1 second; FVC, forced vital capacity; RV, residual volume; TLC, total lung capacity; d, day.

For mechanistic reasons of different target pathways, the mAb group was further stratified into those with anti-IL-5 (mepolizumab) or anti-IL-5R (benralizumab) treatment (n=4) and those with other mAb (n=5) (dupilumab or omalizumab) (Table 2).

Except one patient, all had received at least three COVID-19-mRNA vaccinations. The study was performed according to the principles of the Declaration of Helsinki 2013 and ethical approval (patients were investigated under Translational Lung Research Centre Heidelberg (DZL) broad consent protocol number S-270/2001; University of Heidelberg). Blood samples and nasal swabs were obtained between March and June 2023 covering an interval of 1–3 months after last spike (S) exposure by either booster dose of the Omicron BA.5 adapted or non-adapted ancestral-strain-specific COVID-19-mRNA vaccine or exposure to PCR-confirmed omicron infection (non-lineage specific). At the time point of sampling XBB variants of Omicron were the most prevalent strains in Germany.²⁵ Peripheral mononuclear blood cells (PBMCs), serum and nasal swab samples were processed according to standardized laboratory procedures (Supplementary Table 1) and stored in liquid nitrogen until use.

Characterization of SARS-CoV-2-Stimulated Lymphocytes by Flow Cytometry

PBMCs were precultured in high concentration ($10 \times 10^6/\text{mL}$) for 24 hours in TexMACSTM medium (Miltenyi Biotec, Bergisch-Gladbach, Germany) to increase sensitivity of expected T cell responses as described elsewhere.²⁶ Afterwards, the cells were either stimulated with $10 \mu\text{g}/\text{mL}$ SARS-CoV-2-spike-(S)-ectodomain containing the recombinant binding site, S1- and S2-antigen (Institute Virion/Serion, Wuerzburg, Germany) or $1 \mu\text{g}/\text{mL}$ anti-CD3/anti-CD28 (BioLegend, London, UK) as positive control while the negative control was left in TexMACSTM, all conditions at 1.5×10^6 cells/mL. After incubation (24 hours at 37°C) in the presence of $10 \text{ ng}/\text{mL}$ recombinant human IL-2 (BioLegend) to expand specific effectors and memory T cells, restimulation was performed with $10 \mu\text{g}/\text{mL}$ S antigen or $10 \text{ ng}/\text{mL}$ staphylococcal-enterotoxin-B as positive control. After overnight incubation with $10 \text{ ng}/\text{mL}$ IL-2 and $5 \mu\text{g}/\text{mL}$ Brefeldin A (Sigma-Aldrich, St. Louis, USA) cells were washed and stained with fluorochrome-labeled antibodies and analyzed by FACSCanto II using FlowJo (BD Biosciences).

Cytokine Analysis

A cytokine panel for Th1 and Th2 responses was measured in the cell culture supernatants, serum samples as well as in Nasorption-FXi swabs according to standardized procedure and manufacturer's instruction (Mucosal Diagnostics, Midhurst, UK) (short: nasal swabs) using LEGENDplexTM kit (BioLegend, HuTh Cytokine panel (12-plex; containing IL-5, IL-4, IL-2, IL-6, IFN- γ , TNF- α , IL-9, IL-13, IL-10, IL-17A, IL-17F, IL-22) according to manufacturer's protocol and analyzed using LEGENDplexTM Data Analysis Software (BioLegend).

Anti-Spike-SARS-CoV-2 IgG and IgA Concentrations and Avidity

Serum anti-spike-IgG concentrations were determined by SERION-ELISA agile SARS-CoV-2-IgG (Institute Virion/Serion) and expressed as binding antibody units (BAU/mL). IgA antibodies against S1-spike protein and receptor-binding-domain-(RBD) were measured in serum and nasal swabs using LEGENDplexTM kit (BioLegend, SARS-CoV-2 Serological IgA panel (2-Plex)). Anti-spike-SARS-CoV-2 IgG avidity was measured using a modified ELISA protocol using ammonium thiocyanate as chaotropic agent²⁷ and expressed as relative avidity index (RAI).

SARS-CoV-2-Neutralization Assay

Neutralization activity against SARS-CoV-2 wuhan-type and omicron variant BA.5 was tested in serum and in nasal swabs with a pseudovirus neutralization assay based on a VSV pseudovirus carrying the SARS-CoV-2 spike protein. To produce VSV pseudoviruses codon-optimized, C-terminally truncated spike genes (D614G mutation and Omicron BA.5 variant) cloned into the pSTZ expression plasmid and transfected into HEK293T cells. After 24 hours, cells were inoculated with VSV-EGFP- ΔG -G, a replication-deficient VSV lacking the VSV-G gene but encoding EGFP. After one hour, the inoculum was removed, cells were washed, and incubated for another 24 hours. Supernatants containing VSV-EGFP- ΔG -S were centrifuged and stored at -80°C . For neutralization assays, pseudoviruses were preincubated with donor sera or nasal swab extracts at varying concentrations before infecting Calu-3 cells. Neutralization activity against

SARS-CoV-2 wild type and Omicron BA.5 was assessed by fluorescence after 18 hours. Serum neutralization was quantified as the inhibitory concentration 50% (IC₅₀), representing the dilution at which infection was reduced by half. IC₅₀ values were calculated using nonlinear regression with GraphPad Prism 9.0 Software.

Statistics

Variables were tested for distribution by Kolmogorov–Smirnov-Test. Kruskal–Wallis-test and Mann–Whitney-*U* test were applied for independent continuous non-parametric variables and Wilcoxon rank test for dependent variables. The Bonferroni correction method was performed in the case of multiple groups for error correction. Dichotomous variables were compared using χ^2 or Fisher exact test. Correlations were analyzed by Spearman rank correlation coefficient. A *p*-value ≤ 0.05 was considered statistically significant.

Results

A schematic representation summarizing the key findings of the study is shown in [Figure 1](#).

Characteristics of Study Group

No significant difference regarding demographic, clinical or infection parameters appeared between the analyzed groups ([Table 1](#) and [Supplementary Tables 1 and 2](#)).

Increased Serum IL-5 Concentrations in Anti-IL-5 Treatments

The baseline cytokine profile was determined in serum and nasal swabs. Serum analysis showed that anti-IL-5 treatments showed higher IL-5 concentrations compared to other mAb or conv ([Figure 2A](#) and [B](#) and [Supplementary Table 3](#)) with no significant differences in the other cytokine concentrations ([Supplementary Figure 1](#) and [Supplementary Table 4](#)). In nasal samples, there was no difference in the concentrations of IL-5 between the groups ([Figure 2C, D](#) and [Supplementary Table 5](#)). However, IL-2 was lower in anti-IL-5 group compared to other mAb group ([Supplementary Figure 2](#)). In conv, nasal concentrations of IL-13 (*p*=0.011), IL-6 (*p*=0.011), IL-10 (*p*=0.008), IL-17A (*p*=0.008) were significantly higher compared to the serum samples of the same individuals. In patients treated with either anti-IL-5 or mAb (defined as treated patients), similarly, nasal concentrations of IL-5 (*p*=0.051), IL-13 (*p*=0.011), IL-2 (*p*=0.008), IL-10 (*p*=0.008), IL-17A and IL-17F (*p*=0.008) as well as IL-4 (*p*=0.021) and IL-22 (0.058) were higher compared to matched serum samples ([Supplementary Figures 1, 2](#) and [Supplementary Tables 4, 5](#)).

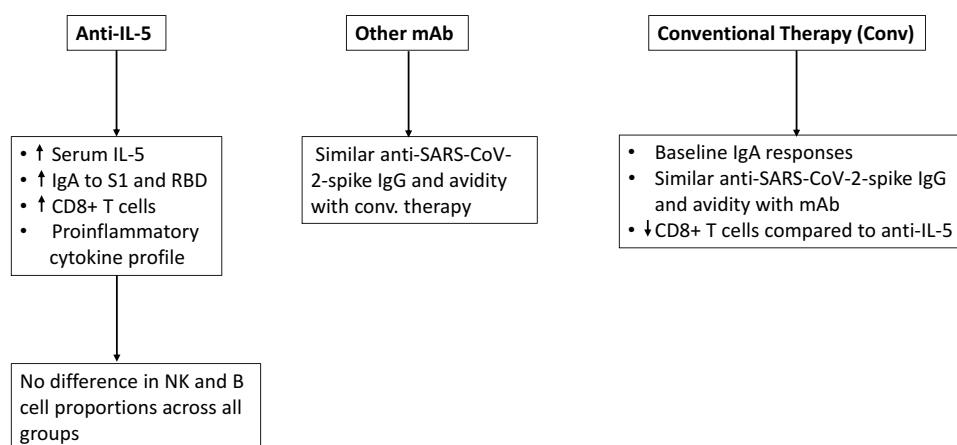
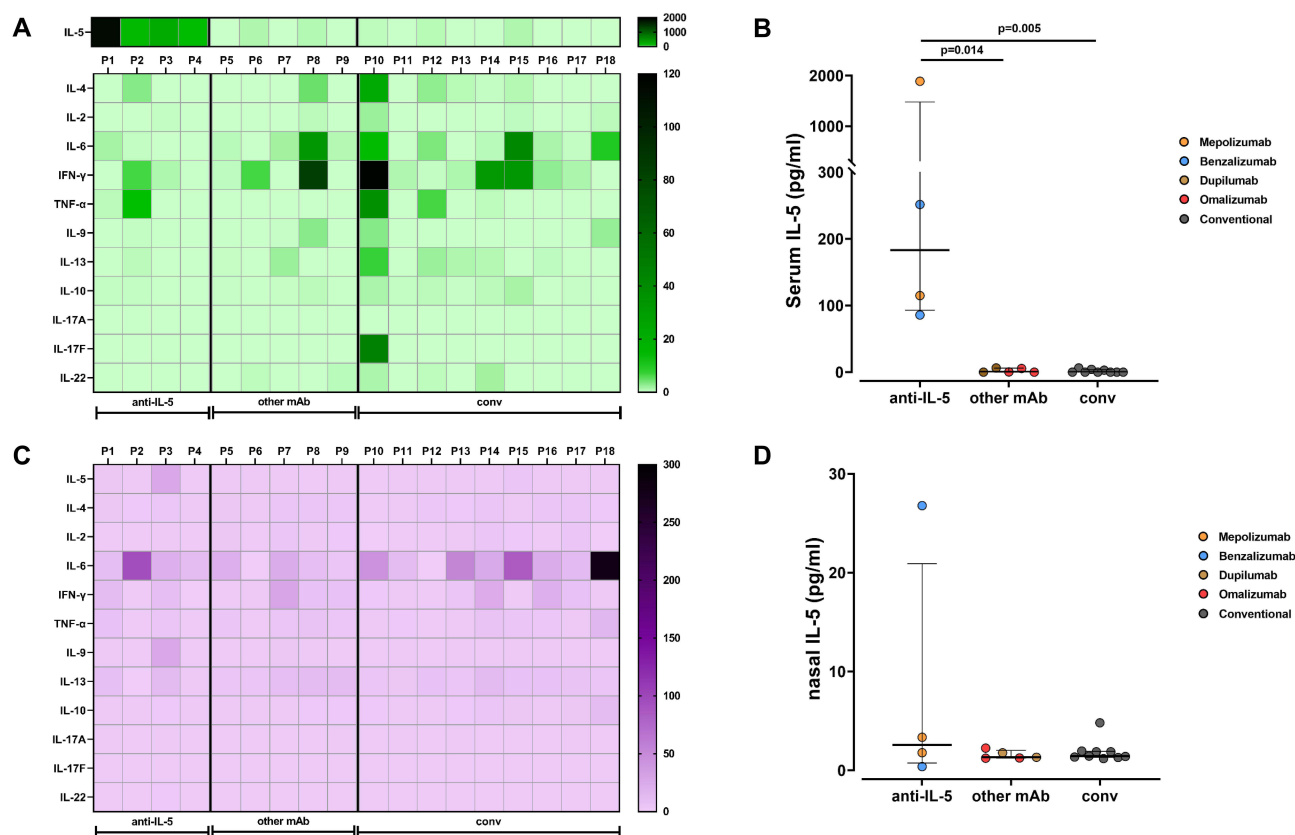


Figure 1 Schematic representation summarizing key immune responses in asthma patients receiving different therapies. Anti-IL-5 treatment is associated with increased serum IL-5, elevated IgA responses to S1 and RBD, and higher proportions of CD8+ T cells compared to conventional therapy. Anti-spike IgG levels and avidity were similar between monoclonal antibody (mAb) treatments and conventional therapy. Additionally, a proinflammatory cytokine profile was observed following in vitro SARS-CoV-2-spike stimulation in the anti-IL-5 group. No significant differences were observed in NK and B cell proportions across groups.



Increased IgA Responses to S1 and RBD in Anti-IL-5 Treatments and Similar Anti-Spike-IgG and Avidity Between mAb and conv

The anti-spike-IgG concentrations (Figure 3A) and their relative avidity index (RAI) (Figure 3B) were determined in serum demonstrating a trend towards higher RAI in anti-IL-5 compared to other mAb ($p=0.086$) (Figure 2B and Supplementary Table 6). In samples of patients treated with anti-IL-5, significantly higher serum and nasal concentrations of anti-S1-IgA were found compared to the other two groups as well as higher serum anti-RBD-IgA compared to any conventional treatment (conv) and higher nasal anti-RBD-IgA compared to other mAb (Figure 3C, D and Supplementary Table 6). No significant difference was seen regarding neutralization activity against ancestral wuhan-type SARS-CoV-2 and against omicron BA.5 variant (Figure 3E and Supplementary Table 6).

Higher Proportions of CD8+ T Cells but Not of NK and B Cells in Asthma Patients Treated with Anti-IL-5 Compared to Conventional Therapy

Proportions of CD4+, CD8+ T cells (Figure 4A), CD16+ NK cells (Figure 4B), CD19+ B cells (Figure 4C), CD138+CD19+ plasma cells (Figure 4D) as well as the proportions of naïve, memory, effector, and Temra CD4+ T cells (Figure 4E), and CD8+ T cells (Figure 4F) were evaluated in PBMCs after in vitro stimulation with SARS-CoV-2-specific spike-protein.

Significant lower proportions of CD8+ T cells were detected in conv- compared to anti-IL-5-treated patients (Figure 4A). No significant differences were found in the proportions of NK cells (Figure 4B) between the groups. Interestingly, the

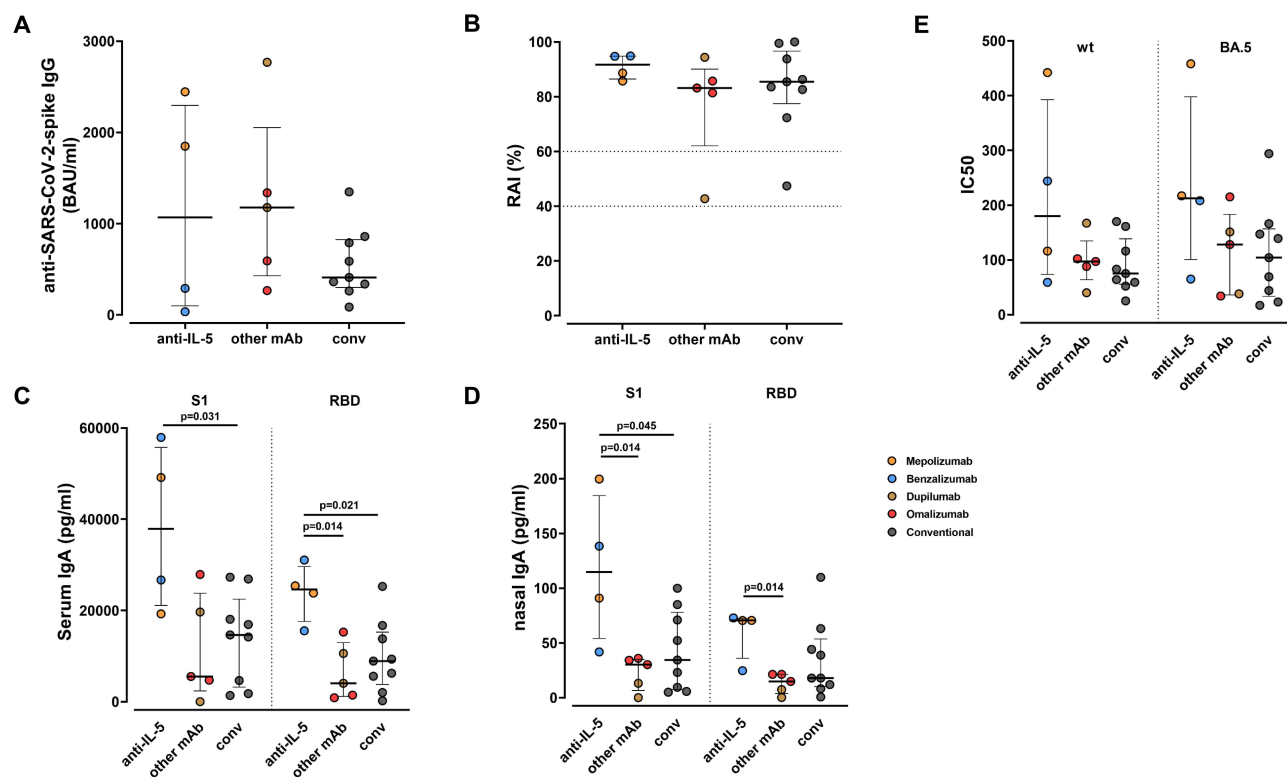


Figure 3 Anti-SARS-CoV-2 spike antibody responses. Antibody levels, avidity, and neutralization capacity were assessed in asthma patients (PI-P18) receiving anti-IL-5 therapy, other mAbs, or conventional treatment (see legend Figure 1). (A) Serum anti-SARS-CoV-2 spike IgG levels (BAU/mL) determined by ELISA. (B) The relative avidity index (RAI) of the anti-SARS-CoV-2 spike IgG, dashed lines defined for low (<40%), intermediate (40%<RAI<60%), and high (≥60%) avidity are indicated. IgA concentrations against spike (S1) and receptor-binding domain (RBD) antigens measured in serum (C) and nasal swabs (D) using LegendPlex™. Neutralization capacity (IC50) of anti-SARS-CoV-2 spike IgG against SARS-CoV-2 wild-type (wt) and omicron (BA.5) variants (E). Data are presented as median ± interquartile range. Statistical significance is indicated by p-values (Kruskal–Wallis and Mann–Whitney U-test).

proportion of CD138+ B cells is significantly higher in conv compared to the other groups (Figure 4D). Consistently, conv patients show higher proportions of B cells compared to patients on anti-IL-5 or other mAb (Figure 4C).

Anti-IL-5-treated lymphocyte samples stimulated with spike-protein demonstrated higher proportions of terminally-differentiated effector memory T cells (Temra) (CD45RA+CD27-) in CD4+ helper T cells compared to other mAb or conv (Figure 4E and Supplementary Table 7), while this difference was not observed in CD8 T cells (Figure 4F).

The IFN γ production following SARS-CoV-2-spike protein stimulation was determined in CD4+ (Figure 5A) and CD8+ T cells (Figure 5B). No significant differences in IFN γ production were found between the groups as well as in the naïve, memory, effector, and Temra CD4+ (Figure 5C). A non-significant relative trend towards higher CD69-expression as indicator for activated CD8+ T cells was seen in anti-IL-5 compared to in other mAb (p=0.086) (Figure 5D and Supplementary Table 8). The proliferative capacity was determined by the expression of Ki67 in CD4 and in CD8 T cells upon stimulation with spike SARS-CoV-2 (Figure 5E) demonstrating no significant difference between the groups.

Regarding cytotoxic capacity, anti-IL-5 treatments demonstrated higher granzyme B expressing CD8+ cytotoxic T cells compared to conv (Figure 5F), but lower perforin expression in CD16+ NK cells (Figure 5G and Supplementary Table 8).

Proinflammatory Cytokine Profile Produced After in vitro SARS-CoV-2-Spike Stimulation

Assessing the Th cytokine profile in supernatants after spike-stimulation, IFN γ and IL-9 production increased in all groups after spike-protein stimulation compared to baseline cytokine production (Figure 6A, B and Supplementary Table 9). Significantly, higher IL-13 concentrations were found in anti-IL-5 compared to other mAb (Supplementary Figure 3 and Supplementary Table 9), as well as IL-2, IFN γ , TNF α , IL-17A, IL-17F and IL-22 (Figure 6, Supplementary Figure 3 and Supplementary Table 9).

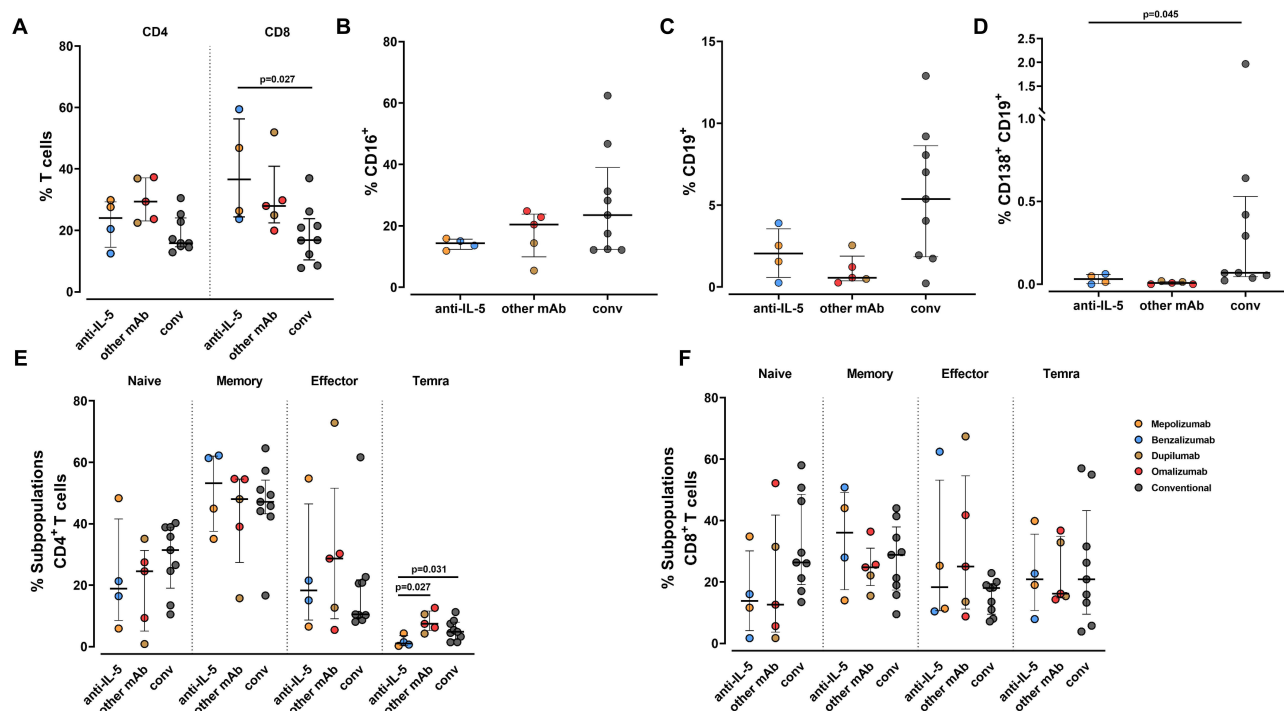


Figure 4 Phenotypic characterization of lymphocytes. Peripheral blood mononuclear cells (PBMCs) from asthma patients with anti-IL-5, other mAb, or conventional therapy (see legend Figure 1) were stimulated in vitro with SARS-CoV-2 spike protein and analyzed by flow cytometry with fluorochrome-labeled antibodies: CD4 as helper T cell marker, CD8 as cytotoxic T cell marker, CD16 as natural killer (NK) cell marker, CD45RA as naive or terminally-differentiated effector T cell marker (Temra), CD27 as naive and early memory T cell marker, CD19 B cell marker and CD138 as plasma cell marker. Data acquisition was performed by FACSCanto II (BD Biosciences) and analyzed in FlowJo™ 10 Software. Frequencies of CD4+ and CD8+ (A) CD16+ (B) CD19+ (C) and CD138+CD19+ (D) were determined. Naive, memory, effector, and terminally differentiated memory T cells Temra were determined in CD4+ (E) and CD8+ T cells (F). Data are presented as median ± interquartile range. Statistical significance is indicated by p-values (Kruskal–Wallis and Mann Whitney U-test).

Correlations of Specific Adaptive Immune Responses

Correlation analysis between demographic parameters, humoral and cellular immune responses was performed in the 9 individuals with monoclonal antibodies (anti-IL-5 and mAb defined as treated patients) and in the 9 individuals with conventional therapy (conv patients). Age negatively correlated with serum IC50 against ancestral Wuhan-type SARS-CoV-2 in treated patients ([Supplementary Figure 4A](#) and [Supplementary Table 10](#)). The concentrations of IL-2 positively correlated with the neutralization capacity against Wuhan-type and BA.5 omicron. Concentrations of serum anti-RBD-IgA and of both serum and nasal anti-S1- and RBD-IgA positively correlated with IL-13 and TNF α .

However, in the 9 individuals of the conv group as well as in the 9 treated individuals receiving mAb, BMI positively correlated with nasal anti-S1-IgA and nasal anti-RBD IgG antibodies ([Supplementary Figure 4B](#) and [Supplementary Table 11](#)), with IC50 against Wuhan-type SARS-CoV-2 and near to significance with anti-spike-IgG concentrations ($p=0.058$). Anti-spike-IgG concentrations positively correlated with avidity and IC50 values against ancestral and omicron SARS-CoV-2.

Avidity correlated with IL-4 production after spike-protein stimulation in conv patients ([Supplementary Figure 4B](#) and [Supplementary Table 11](#)). In treated patients, IL-10, IL-17A, and TNF α positively correlated with nasal anti-S1. In both groups, spike-stimulated subpopulations of T cells and NK cells did not significantly correlate with humoral immunity parameters. Proportions of activated CD69-expressing CD4+ and CD8+ T cells correlated with each other in treated patients ([Supplementary Figure 4C](#) and [Supplementary Table 12](#)). Proportions of IFN γ -producing and Ki67+ CD4+ T cells significantly correlated with the proportions of IFN γ and Ki67 expressing memory CD4+ T cells. In contrast, circulating Ki67+ effector CD4+ T cells correlated with the concentrations of IFN γ -producing CD4+ T cells in conv ([Supplementary Figure 4D](#) and [Supplementary Table 13](#)).

Following in vitro stimulation with SARS-CoV-2-spike-protein, in treated patients, concentrations of IL-2 and IL-13 positively correlated, as well as IFN γ , TNF α , IL-17A, IL-17F, IL-22, and IL-10 ([Supplementary Figure 4E](#) and

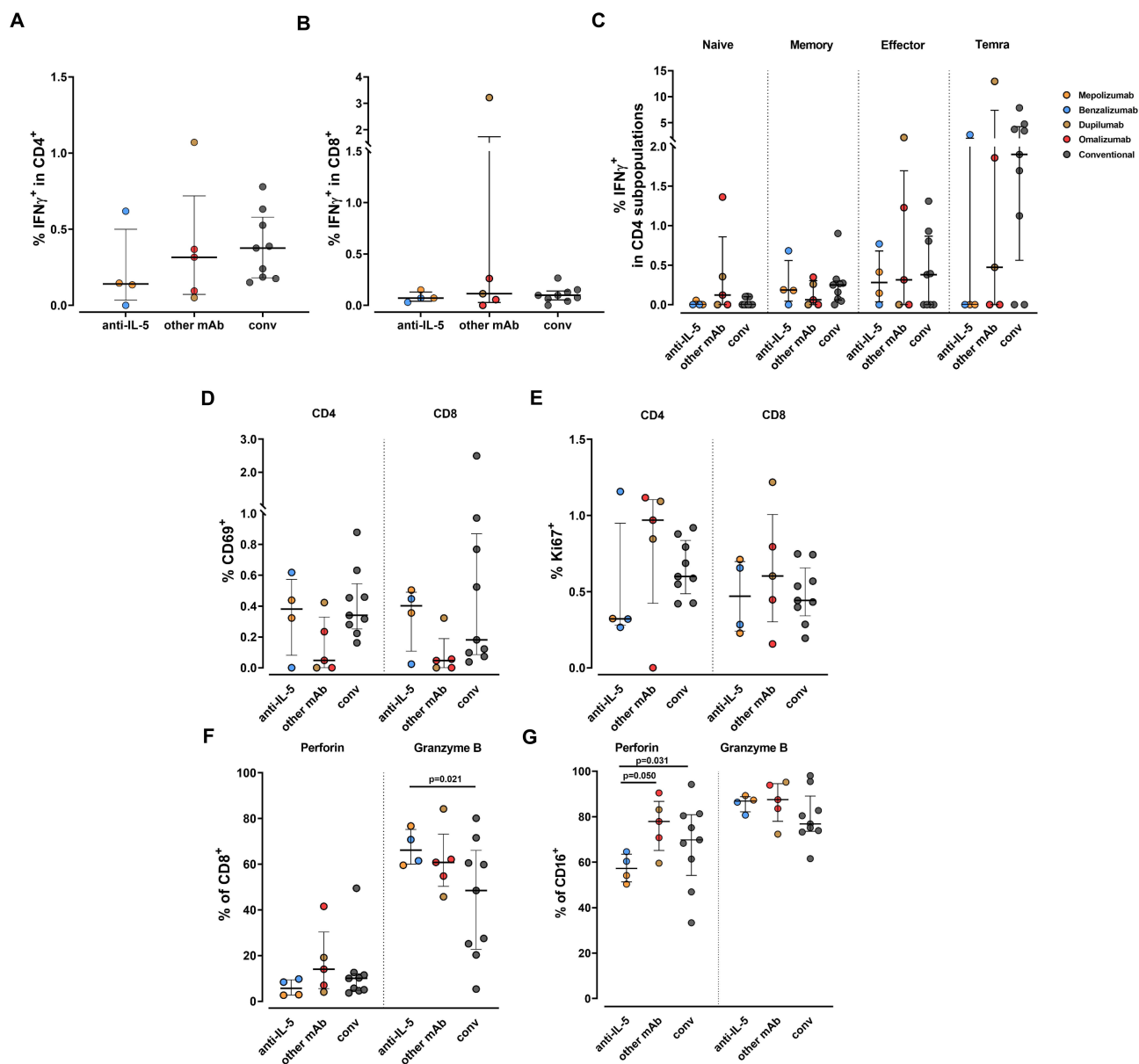


Figure 5 T cell response to SARS-CoV-2 spike protein. Intracellular detection of IFN γ was determined following PBMCs in vitro stimulation with SARS-CoV-2-spike protein in (A) CD4 $^{+}$, (B) CD8 $^{+}$ and naive, memory, effector, and terminally differentiated memory T cells Temra CD4 $^{+}$ (C) defined by the expression of CD45RA as naive or terminally-differentiated effector T cell marker (Temra) and CD27 as naive and early memory T cell marker. Activated cells characterized by the expression of CD69 (D) were measured in CD4 $^{+}$ as T helper cells and CD8 $^{+}$ as cytotoxic T cells (D). Proliferative (E) Ki67 $^{+}$ CD4 $^{+}$ and CD8 $^{+}$ as well as the production of perforin and granzyme B to determine cytotoxic profile were assessed in (F) CD8 $^{+}$ and CD16 $^{+}$ as natural killer (NK) cell marker (G) cells following in vitro stimulation with SARS-CoV-2-spike. Data acquisition was performed by FACSCanto II (BD Biosciences) and analyzed in FlowJoTM 10 Software. Data are presented as median $\pm$ interquartile range. Statistical significance is indicated by p-values (Kruskal–Wallis and Mann Whitney U-test).

[Supplementary Table 14](#)). Lymphocyte samples of the conv group following in vitro stimulation showed a negative correlation between the concentrations of IL-5 and IL-9, while a positive correlation was observed between TNF α and IL-17A ([Supplementary Figure 4F](#) and [Supplementary Table 15](#)).

Discussion

The present study aimed to determine the immunogenicity against SARS-CoV-2-specific spike-protein in asthma patients on mAb therapy or conv therapy and to identify mediators of cellular T cell reactivity in correlation to humoral immunity parameters and B cell reactivity. The investigations revealed that patients on IL-5-targeting treatments showed relatively

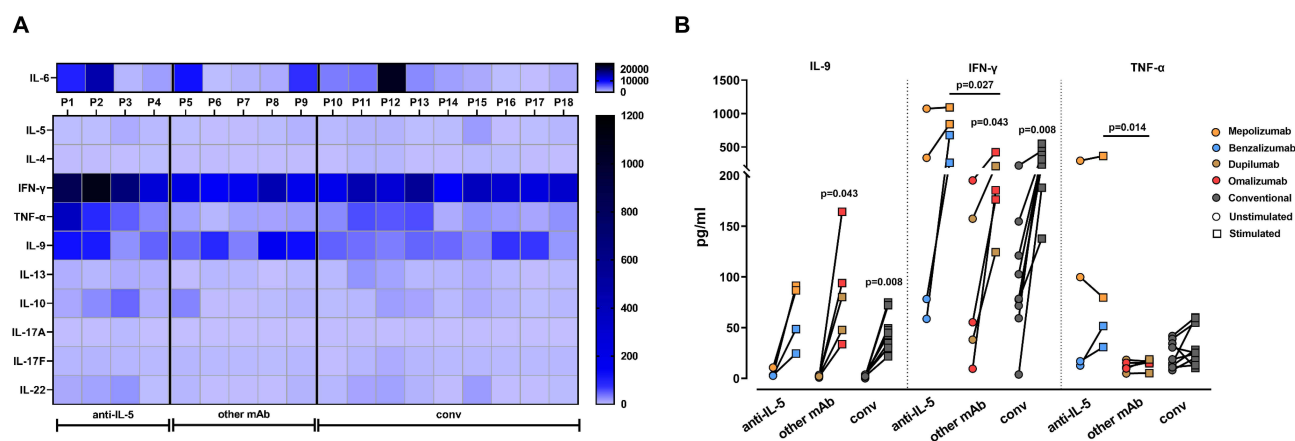


Figure 6 Cytokine production following in vitro stimulation with SARS-CoV-2-spike protein. The concentration (pg/mL) of IL-5, IL-4, IL-6, IFN-γ, TNF-α, IL-9, IL-13, IL-10, IL-17A, IL-17F, IL-22 were determined in supernatants upon SARS-CoV-2-spike stimulation using a bead based cytokine assay (LegendPlex™) in asthma patients treated with anti-IL-5, other mAb, and conv therapy (see legend Figure 1). **(A)** Cytokine profiles for all patients (P1–P18) following spike protein stimulation. **(B)** Cytokine levels of IL-9, IFN-γ, and TNF-α expressed in pg/mL pre and post stimulation with SARS-CoV-2-spike are shown. Patients were grouped by treatment (anti-IL-5, other mAb, conv). Statistical significance is indicated by p-values (Wilcoxon rank test).

higher serum and nasal IgA antibodies against spike-protein components, such as S1 and RBD, compared to patients with other mAb or conv therapy. Serum anti-RBD-IgA concentrations were associated with significantly higher IL-13 and TNFα production after spike-protein stimulation of PBMCs. IL-4 correlated with higher anti-spike-IgG avidity in this group. Thus, asthma patients treated with monoclonal antibodies against IL-5 or its receptor to restore dysbalanced Th2-driven inflammatory responses may benefit from the targeted therapy regarding development of specific antibody responses resulting in higher mucosal anti-spike IgA compared to conventionally treated patients. The findings from this study indicate that immunomodulation by targeting IL-5 does not impair specific adaptive immune responses and instead allows to develop quantitatively better IgA responses than in patients with conventional therapy or therapies targeting the IL-4/IL-13 axis or directly IgE antibodies. Although IgA were not distinguished into IgA1 or IgA2 type or into monomeric or dimeric structured molecules, the relevance of nasal IgA may be suggested regarding defense against SARS-CoV-2.¹³

The baseline accumulation of serum IL-5 found in our anti-IL-5-treated patients may be explained by the lack of consumption of IL-5 by cells with blocked IL-5 receptor by benralizumab or by the neutralization of free IL-5 which may be still detectable by bead-based antibodies from the multiplex assay targeting different epitopes compared to mepolizumab. IL-5, commonly produced by Th2 cells, plays an important role in the maintenance of B cells and IgA production, also related to the survival of antibody-secreting cells.²⁸ In mucosal areas, IL-5 may indirectly influence the IgA-production through promoting mucosal B cell survival²⁹ which may explain the increased concentrations of anti-S1- and anti-RBD-IgA in our cohort. Previous studies reported that, in general, antibody concentration and neutralization after SARS-CoV-2 vaccination do not differ among asthma patients with or without biological treatment^{30,31} which goes in line with our findings that anti-spike-IgG responses and avidity was not significantly different in patients on biologics compared to those on conventional therapy. Moreover, a trend towards higher avidity was seen in anti-IL-5 treatments. IgG antibodies of anti-IL-5-treated patients against Wuhan-type and omicron BA.5 variant had a trend towards better neutralizing capacity compared to the other two groups.

T cell responses have been described as a second pillar of adaptive immunity against viral infections, such as SARS-CoV-2. An expansion of CD8+ T cells was shown in anti-IL-5-treated patients after spike-protein stimulation compared to other mAb or conv, with a higher expression of the cytotoxic molecule granzyme B. These findings may implicate a CD8-driven response in anti-IL-5 with lower terminally-differentiated-effector-memory CD4+ T cells directed against spike-protein. It has been discussed that generally the number of circulating active and proliferating SARS-CoV-2-spike-specific T cells is low in asthma patients, which may be associated with T cell exhaustion induced by asthma contributing to a defective capacity for viral protection.³² Furthermore, a poor virus clearance found in asthma patients may be related

to an impaired T cell response due to allergic sensitization of the T cells.³³ Asthma patients often exhibit dysregulated cytokine responses, leading to an impaired T cell-mediated immune response against viral infections³² as the imbalance between pro-inflammatory and anti-viral cytokines is a key factor to define T cell dysfunction with a bias towards Th2 cytokines, such as IL-4, IL-5 and IL-13.³⁴

In our cohort, stimulation with spike-protein clearly induced secretion of IFN γ , a key cytokine of the Th1 response, and remarkably, production of IL-9 in all groups. IL-9-producing cells are considered as distinct T helper cell subset which is induced by combination of IL-4 and TGF- β ³⁵ but closely related to Th2 with a role in the progression of allergic asthma.^{36,37} Additionally, IL-9-secreting cells can be induced under similar differentiation pathways as Th17 cells.³⁸ In the context of viral infections, IL-9 has been shown to play a role in the clearance of viral infections as shown for respiratory syncytial virus (RSV).³⁹ However, it has been demonstrated that IL-9 promotes the severity of SARS-CoV-2 infection by exacerbating lung inflammation by the suppression of anti-viral gene function on T cells during infection.⁴⁰ In our cohort, with focus on in vitro recall reaction to spike-protein in COVID-19-vaccinated asthma patients, strong IL-9, IFN γ and TNF α responses after spike-protein stimulation were seen in the two patients on mepolizumab, suggesting an effect of blocking IL-5 on the production of these cytokines. Baseline Th1 cytokine production and responses after spike-protein stimulation correlated in all patients treated with targeted therapies by mAb. This may indicate a partial reconstitution of the cytokine balance in patients treated with mAb against dysbalanced Th2-driven responses, which may explain a potential contribution to improved class switch towards IgA and the significant better IgA responses particularly in anti-IL-5 treated patients.

Recently, investigation of the humoral and cellular immune response in patients with asthma or atopic dermatitis on benralizumab, dupilumab or mepolizumab showed lower immunity after two doses of COVID-19 mRNA vaccination.⁸ In contrast, all patients of our cohort except one female person exhibited at least three S-protein exposures by vaccination with either an omicron BA.5-adapted COVID-19 mRNA vaccine or with non-adapted mRNA vaccine encoding for ancestral SARS-CoV-2 S-protein. Thus, repeated exposures to S-protein may improve the immunity although treated by cytokine-targeting biologics.

Despite the suggested role of Th responses in the modulation of anti-S reactivity, macrophages as well as tissue monocytes maturing into antigen-presenting dendritic cells have to be acknowledged as prominent source of IFN γ and other proinflammatory cytokines but were not investigated in this cohort. Macrophages have been identified as critical players in hyperinflammation in severely affected patients with COVID-19⁴¹ as well as in modulating specific anti-viral responses. In contrast, alveolar macrophages may also be altered by specific antibody responses against S protein.⁴²

However, our study is limited by the small groups and the high heterogeneity regarding number of spike exposures by either the COVID-19-mRNA vaccine or SARS-CoV-2 breakthrough infection and by the high inter-individual variance regarding baseline cytokines and cytokine responses towards specific spike-protein stimulation as well as that the cohort was not exclusively including eosinophilic asthma. Despite these drawbacks the present study indicates a significant cellular and humoral immune response to SARS-CoV-2 spike-protein in all asthma patients of this study independently of type of systemic therapy demonstrating balanced cytokine responses regarding spike-induced Th1- and Th2-profiles.

Conclusion

To summarize, the findings indicate that patients with asthma independently of type of therapeutical disease control are able to mount a specific adaptive immune response to SARS-CoV-2 spike-protein exposures which are characterized by Th1-driven and Th2-driven responses. Particularly patients with anti-IL-5 treatments showed a significantly higher response by serum and mucosal anti-S1 and anti-RBD-IgA compared to IL-4/IL13-targeting, anti-IgE or conventional therapies. These results suggest that immunogenicity of COVID-19-mRNA vaccines or infection-induced spike-exposures is equivalent between asthma patients on monoclonal antibodies compared to those treated with conventional therapy. The findings may be of particular importance for deducing potential effects of currently approved mRNA vaccine encoding for prefusion-protein of RSV or immunogenicity of future mRNA vaccines targeting other respiratory viruses in asthma patients on monoclonal antibodies.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used DeepL (DeepL SE, Cologne, Germany) in order to improve language only. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Abbreviations

mAb, Monoclonal antibody; Th, T Helper; IL, Interleukin; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; LAMA, Long-acting muscarinic antagonist; LABA, Rapid onset long-acting beta-2-agonist; conv, Conventional; PBMCs, Peripheral blood mononuclear cells; Ig, Immunoglobulin; RBD, recombinant-binding-domain; ICS: inhaled corticosteroids; OCS, Oral corticosteroids; NK, Natural killer cells; Temra, terminally-differentiated effector memory T cell; BAU, Binding antibody units.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

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