

**INVESTIGATION OF THE SARS-CoV-2 INDUCED
IMMUNE RESPONSES IN RHEUMATOID
ARTHRITIS AND HEALTHCARE WORKERS**

PhD thesis

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1. Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by persistent synovial inflammation, progressive joint destruction and systemic immune dysregulation. Affecting approximately 0.5–1% of the adult population, RA arises from complex interactions between genetic predisposition, environmental factors and aberrant immune responses. The disease is driven by sustained activation of innate and adaptive immune pathways, with key roles for pro-inflammatory cytokines including tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6) and interleukin-1 β (IL-1 β). In addition to promoting chronic inflammation, these mechanisms impair host defense, resulting in increased susceptibility to infections. This vulnerability is further exacerbated by immunosuppressive therapies, which are essential for disease control but can compromise immune competence. These interconnected mechanisms and their clinical consequences are illustrated in Figure 1.

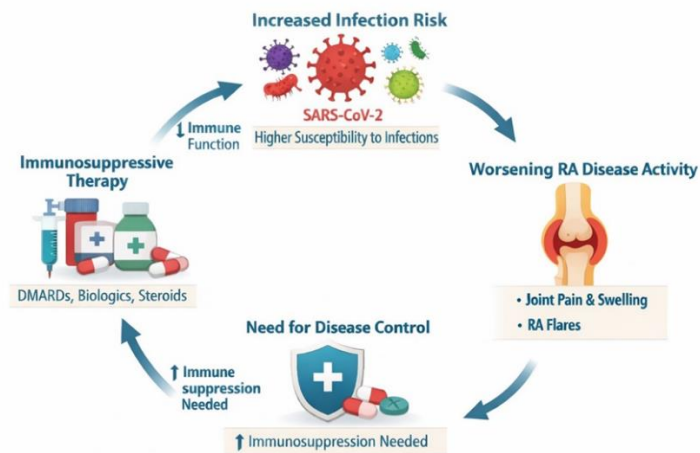


Figure 1. Interaction between immunosuppression, infection risk and disease activity in RA. Immunosuppressive therapies might reduce immune competence and increase susceptibility to infections, including SARS-CoV-2. Infections may trigger systemic inflammation and disease flares, leading to intensified immunosuppressive treatment and perpetuating a self-reinforcing cycle. *The figure was created using OpenAI GPT-5.3 based on the author’s conceptual design.*

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 2019 and the subsequent

coronavirus disease 2019 (COVID-19) pandemic posed unprecedented global health challenges. Beyond respiratory pathology, SARS-CoV-2 infection may induce profound immunological alterations, including cytokine dysregulation, lymphocyte dysfunction and autoimmune phenomena. These effects are particularly relevant in individuals with pre-existing immune-mediated diseases such as RA, where immune homeostasis is already disrupted.

RA and COVID-19 share overlapping immunopathological features, including excessive cytokine production and dysregulated T- and B-cell responses. These similarities raise critical questions regarding susceptibility to infection, disease severity and vaccine responsiveness in RA patients. Furthermore, immunomodulatory therapies may differentially influence antiviral immunity and vaccine-induced protection.

Healthcare workers (HCWs) represent another key population during the pandemic due to sustained occupational exposure to SARS-CoV-2. Their close

interaction with vulnerable patient groups, including immunocompromised individuals, creates a bidirectional transmission interface. Understanding immune responses in both RA patients and HCWs is therefore essential for optimizing vaccination strategies and infection control.

In this context, comprehensive evaluation of both humoral and cellular immune responses following SARS-CoV-2 infection and vaccination is crucial. This thesis investigates these responses in RA patients and HCWs, with particular emphasis on determinants of immune response magnitude and the longitudinal dynamics of immunity.

2. Objectives

The primary objective of this thesis was to investigate the SARS-CoV-2-induced immune responses in RA patients and in HCWs.

The specific aims were:

- to characterize the humoral and cellular immune responses to SARS-CoV-2 infection and COVID-19 vaccination in RA patients and healthy individuals,
- to identify the clinical and therapeutic factors influencing the SARS-CoV-2-induced immune responses,
- to evaluate the longitudinal dynamics and durability of the SARS-CoV-2-specific immune responses over time,
- to assess the immunogenicity and effectiveness of primary and booster BNT162b2 vaccination in HCWs.

3. Methods

This work was based on observational cohort studies involving RA patients and HCWs.

3.1 Study populations

RA patients were recruited from the rheumatology outpatient department of Semmelweis University. Eligible participants were adults diagnosed according to the 2010

ACR/EULAR classification criteria, with documented SARS-CoV-2 infection and/or COVID-19 vaccination. A total of 109 patients were included in the baseline analysis and 84 patients in the longitudinal follow-up study. A control group of healthy individuals was also enrolled. RA disease activity was generally low. The majority of patients received conventional and/or targeted immunomodulatory treatment, including methotrexate, TNF- α inhibitors, IL-6 inhibitor and anti-CD20 therapy.

HCWs were included in a prospective cohort study following BNT162b2 mRNA vaccination. Of 345 vaccinated individuals, 294 were included in the final analysis. Participants were stratified based on prior SARS-CoV-2 infection status and followed for up to 18 months.

3.2 Immunological assessment

Humoral immune responses were evaluated using validated immunoassays measuring antibodies against the SARS-CoV-2 spike protein receptor-binding domain and nucleocapsid protein. Quantitative and semi-quantitative methods were applied to determine antibody levels and seropositivity.

Cellular immune responses were assessed using an interferon- γ (IFN- γ) release assay (QuantiFERON), measuring antigen-specific T-cell responses following stimulation with SARS-CoV-2-derived peptides.

3.3 Study design

Both cross-sectional and longitudinal analyses were performed. Follow-up investigations assessed immune response dynamics over time, including the effects of booster vaccination and SARS-CoV-2 infection. Defined time intervals enabled evaluation of immune kinetics and response durability.

3.4 Statistical analysis

Descriptive statistics were used to summarize demographic and clinical characteristics. Group comparisons were performed using appropriate parametric and non-parametric tests.

Multivariable regression models were applied to identify determinants of immune responses, including age, sex, treatment regimens and time since immunization.

Antibody levels were log-transformed where appropriate. Longitudinal analyses included estimation of antibody decay and correlation analyses between humoral and cellular responses. All analyses were conducted using established statistical software.

4. Results

4.1 SARS-CoV-2-specific humoral immune responses

SARS-CoV-2-specific antibody responses were detected in the majority of participants. In the baseline analysis, 94% of RA patients and 100% of healthy controls exhibited positive anti-spike antibody responses. However, antibody concentrations were consistently lower in RA patients compared with controls (Figure 2A).

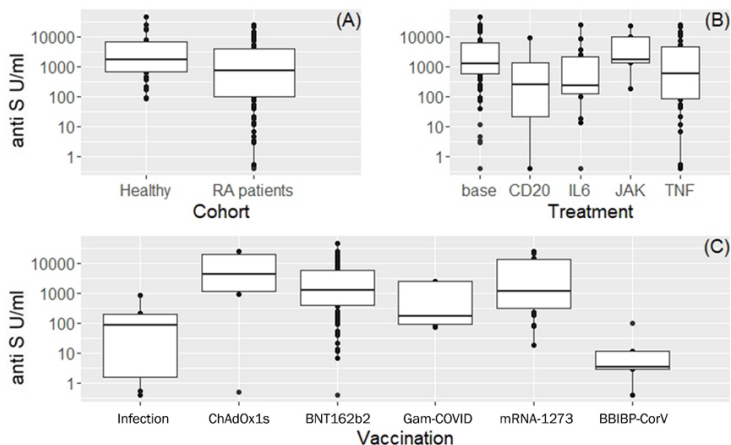


Figure 2. (A) SARS-CoV-2-specific anti-spike (S) antibody levels in RA patients and healthy controls. Antibody concentrations are presented as box plots, demonstrating significantly lower humoral immune responses in RA patients compared with healthy individuals. (B) Anti-spike (S) antibody levels according to targeted treatment groups. The reference group comprises healthy controls and RA patients treated with conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) and/or glucocorticoids (GCS). (C) Anti-S antibody levels following different COVID-19 vaccines administered in Hungary among RA patients and healthy individuals.

Multivariable analysis demonstrated that antibody levels declined significantly over time, with an estimated reduction of approximately 25% per month following COVID-19 vaccination or SARS-CoV-2 infection. Increasing age was also associated with reduced antibody levels. Immunosuppressive therapies had a substantial impact on humoral responses. B-cell-depleting therapy (rituximab) resulted in a marked reduction in antibody levels, while TNF- α inhibitors and IL-6 inhibitor were also associated with significantly decreased responses (Figure 2B). Our study showed that SARS-CoV-2-specific antibody levels were many times higher after mRNA-1273 and BNT162b2 vaccination compared to previously infected but unvaccinated individuals (Figure 2C). We found anti-S levels were higher in the presence of anti-CCP.

In the longitudinal analysis, antibody levels increased significantly in both RA patients and controls over time (Figure 3A), particularly following booster vaccination or SARS-CoV-2 infection. Median antibody concentrations

increased several-fold compared with baseline values, although levels remained lower in RA patients.

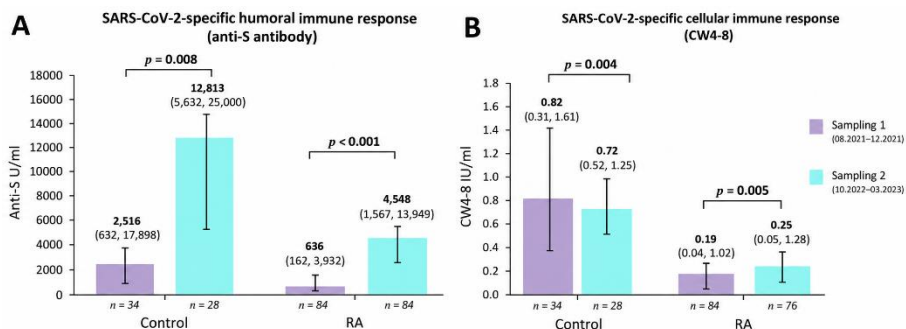


Figure 3. Comparison of SARS-CoV-2-specific humoral and cellular immune responses between healthy controls and patients with rheumatoid arthritis (RA). **(A)** Anti-spike (S) antibody concentrations (U/mL) were significantly lower in RA patients than in healthy controls at both sampling time points (Sampling 1: $p = 0.008$; Sampling 2: $p < 0.001$). **(B)** SARS-CoV-2-specific cellular immune responses, assessed by whole-virus-stimulated CD4⁺ and CD8⁺ T-cell responses (CW4–8), were also significantly reduced in RA patients at both time points (Sampling 1: $p = 0.004$; Sampling 2: $p = 0.005$). *The figure*

was created using OpenAI GPT-5.3 based on the author's conceptual design.

4.2 SARS-CoV-2-specific cellular immune responses

At baseline, SARS-CoV-2-specific T-cell responses, assessed by IFN- γ production, were detectable in both RA patients and healthy controls, but were generally lower in RA patients. Based on our results, increasing age, longer time since antigenic exposure and use of anti-CD20 therapy profoundly suppressed cellular immunity.

During follow-up, T-cell responses remained detectable over time in both groups but were consistently reduced in the RA group (Figure 3B). Based on our findings the enhanced antibody response, along with a durable SARS-CoV-2-specific T-cell response, may help reduce severe COVID-19 cases in the population (Figure 4A, 4B).

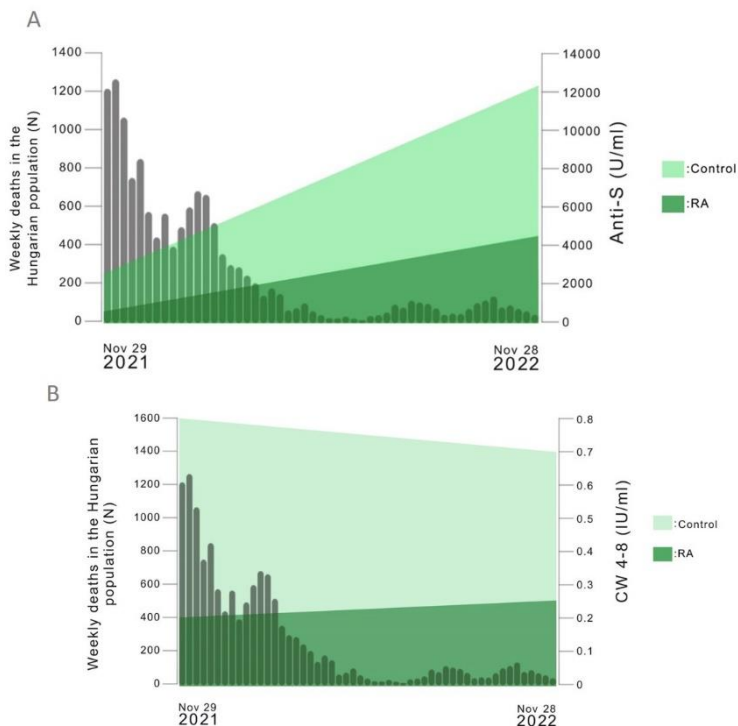


Figure 4. (A) Weekly COVID-19 mortality in Hungary plotted against median anti-spike (S) antibody levels over time by study group. Medians from Sampling 1 and 2 are connected. **(B)** Weekly COVID-19 mortality in Hungary plotted against whole COVID virus-specific CD4+ and CD8+ T-cell responses (CW 4–8) over time by study group, with Sampling 1 and 2 medians connected.

4.3 HCWs cohort

In HCWs, BNT162b2 vaccination induced robust humoral immune responses. Individuals with prior SARS-CoV-2 infection demonstrated significantly higher antibody responses after the first vaccine dose compared with infection-naïve individuals. In contrast, infection-naïve participants required two vaccine doses to achieve comparable antibody levels.

During long-term follow-up, SARS-CoV-2 infection occurred in 41% of participants, predominantly during waves associated with viral variants. Infections were generally mild. Booster vaccination significantly increased antibody levels and was associated with reduced disease severity, although it did not fully prevent SARS-CoV-2 infection.

5. Conclusions

Major conclusions of the study:

- SARS-CoV-2-specific immune responses are highly impacted by host factors, prior SARS-CoV-2 infection, COVID-19 vaccination status and immunosuppressive therapy.

- RA patients show attenuated humoral and cellular immune responses compared with healthy controls.
- Reduced immune responses in RA are associated with older age, longer time since antigenic exposure and immunosuppressive treatment.
- TNF- α and IL-6 inhibitors primarily impair humoral responses, whereas anti-CD20 therapy profoundly suppresses both humoral and cellular immunity.
- Longitudinal data indicate that humoral immunity remains dynamic, while T-cell responses are relatively stable.
- In HCWs, BNT162b2 vaccination is safe and highly immunogenic.
- Prior SARS-CoV-2 infection strengthens COVID-19 vaccine responses and booster doses increase antibody levels and can reduce the disease severity.
- These findings support personalized, therapy-guided vaccination and immune monitoring to improve protection and limit severe outcomes.

6. Bibliography of the candidate's publications

Publications related to the PhD thesis:

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**These authors have contributed equally to this work.*

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