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REVIEW ARTICLE

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Anti-SARS-CoV-2 mRNA vaccines as inducers of humoral response against apolipoprotein A-1?

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Abstract

Background: COVID-19 and some anti-SARS-CoV-2 vaccines trigger a humoral autoimmune response against a broad range of endogenous components, which may affect recipients' prognosis in predisposed individuals. Autoantibodies directed against apolipoprotein A-1 (AAA1 IgG) the major protein fraction of High Density Lipoprotein have been shown to be raised in COVID-19 and in rheumatoid arthritis (RA) patients and other populations where they have been associated with poorer outcomes. We wanted to assess the impact of anti-SARS-CoV-2 mRNA-based vaccination on AAA1 autoimmune biomarkers in RA patients.

Methods: 20 healthy controls and 77 RA mRNA-based vaccinated patients were collected at baseline, 3 weeks after the first vaccination, 2 and 8 weeks after the second vaccination. AAA1 and SARS-CoV-2 serologies were measured by immunoassays. Systemic and local symptoms occurring during the vaccination protocol were recorded.

Results: mRNA-based vaccination induced a significant increase in median AAA1 IgG levels in both healthy controls and RA patients overtime. However, in both populations, these medians trend did not translate into significant increase in AAA1 IgG seropositivity rates despite evolving from 5 to 10% in healthy controls, and from 9 to 12.9% in RA patients. No associations were retrieved between AAA1 IgG and symptoms of any kind during the vaccination protocol.

Conclusions: mRNA-based vaccination seems to induce a light AAA1 IgG response in immunocompetent individuals within 2 months after the last injection. Although we did not observe any warning signs, the formal demonstration of the harmlessness of such biological warrants further studies.

KEYWORDS
anti-SARS-CoV-2 mRNA vaccines, apolipoprotein A-1, autoantibodies

1 | INTRODUCTION

Vaccine-induced antibody-dependent enhancement and/or autoimmune mechanisms have been reported to account for rare complications following poliovirus and influenza A (H1N1) vaccination programmes, leading to documented and sometimes permanent neurological symptoms despite undisputed overall risk-benefit ratios.[1–3] Recent evidence indicate that both COVID-19 and some anti-SARS-CoV-2 vaccines (especially ChAdOx1 nCoV-19) trigger a humoral autoimmune response against a broad range of endogenous components, which may affect recipients' prognosis in predisposed individuals.[4–6] Alleviating such concerns in the actual context of the unprecedented worldwide vaccines roll-out where standard experimentation procedures have been relaxed[1,7] may facilitate vaccination adherence. To this end, assessing the impact of anti-SARS-CoV-2 vaccination on autoimmune biomarkers as an additional objective measure of anti-SARS-CoV-2 vaccines' safety could be of valuable help to decrease the current vaccine hesitancy. Among autoantibodies of interest, those directed against apolipoprotein A-1 (apoA-1)—the major protein fraction of high-density lipoprotein (HDL)—are of particular interest as they have been shown to be raised in COVID-19[8] in RA and other populations where they have been associated with poorer outcomes (reviewed in Ref. 9).

Here, we report the impact of anti-SARS-CoV-2 vaccination in 21 healthy controls and in 77 rheumatoid arthritis (RA) patients on the development of autoantibodies against apoA-1 (AAA1 IgGs) up to 8 weeks after the second injection and analysed their associations with anti-spike serological response and the occurrence of local and systemic symptoms during vaccination.

2 | MATERIAL AND METHODS

2.1 | Study population

As an extension of previous study published elsewhere,[10] healthy controls (healthcare workers) and RA patients were eligible for vaccination according to the Swiss federal regulations and were enrolled in the RECOVER study, a nonrandomized, prospective, observational trial approved by the Ethical Committee of St Gallen, Switzerland. The RECOVER trial is registered by the Business Administration System for Ethics Committees (number2021-00156). Reporting of the study conforms to broad EQUATOR guidelines.[11] None of the patients or controls reported symptoms suggestive of COVID-19 at baseline or during the observation period, and none had a positive SARS-CoV-2 antigen or RT-PCR test. Four patients had antibodies to nucleoprotein at baseline consistent with previously unnoticed COVID-19, and these patients were excluded from further analysis. Written consent was obtained from all patients before inclusion. Nine patients received two doses of the mRNA-1273 vaccine (Moderna), and all others received two doses of the BNT162b2 vaccine (Pfizer–BioNTech).

2.2 | Sampling and biochemical analyses

Serum samples were collected at baseline, 3 weeks after the first vaccination, 2 and 8 weeks after the second vaccination, processed and stored at −80°C until analyses. Quantitative antibody testing was done using the Roche Elecsys Anti-SARS-CoV-2 spike subunit 1 (S1) assay measuring total antibodies to SARS-CoV-2 spike protein 1 according to manufacturer's instruction with an anti-S1 seropositivity cut-off set at 0.8 U/ml. AAA1 IgG were measured using an extensively validated in house ELISA allowing the detection of autoantibodies against native and lipid-free apoA-1.[8] The conventional AAA1 IgG seropositivity cut-off was prospectively defined and usually set at an optical density measured at 405 nanometres (OD_{405}) >0.64, corresponding to the 97.5th percentile of AAA1 IgG levels obtained on healthy blood donors.[8] At an intermediate of 0.6 OD_{405}, the interassay CV was 9% ($n = 5$), and the intra-assay CV was 5% ($n = 5$).

2.3 | Symptom assessment during vaccination protocol

Systemic and local symptoms occurring during the vaccination protocol were recorded. The assessor was blinded to the biochemical results during the follow-up visits. Local symptoms consisted in pain and/or erythema at the injection site, systemic symptoms consisted of generalized myalgias, arthralgias, fever, fatigue, cutaneous lesions, lymphadenopathy or headache. Local and systemic symptoms were rated according to the patient`s perception from 0 to 10 with 10 indicated the highest severity.

2.4 | Statistics

Results were reported as proportion, mean (± standard deviation [SD]) and median, interquartile range (IQR), unless stated otherwise. Differences between groups were calculated using Fisher's exact bilateral test, *t* test or the Mann-Whitney test for independent categorical and continuous variables when appropriate. The nonparametric Friedman ANOVA test was

used to assess median differences evolution of AAA1 IgG levels over time. These differences were further challenged by taking into account the analytical imprecision-derived least significant change (LSC), representing the smallest significant detectable difference between two measurements conventionally defined as 1.96 × √2 × coefficient of variation (CV).[12] Taking into account the interassay CV of 9% for AAA1 IgG, the LSC was 0.06 OD_{405nm}. Spearman correlation was used to assess correlations between the variables. All analyses were performed using Statistica software (version 13.5.0.17, TIBCO Software Inc.). Statistical significance was defined as $p < 0.05$.

3 | RESULTS

The healthy controls and RA patients' characteristics are provided in Table 1, as well as differences between these two groups. Baseline AAA1 seropositivity rate was 5% (1/20) in healthy donors and 9% (7/77) in RA, without significant difference observed for AAA1 IgG levels between these two groups. No associations between baseline individuals/patients characteristics and AAA1 IgG levels were retrieved. Intervals between the first and second vaccine dose and the intervals between vaccination and serum sampling were comparable between patients and controls (Table 1).

Table 1 shows that vaccination-related symptoms (local and systemic) at first and/or second injection, occurred frequently and at similar rates in healthy controls compared to RA patients (70.0% vs 74.0%) (14/20) of and 74% (57/77), without any further difference considering systemic or locals symptom between these two groups.

As shown in Figure 1, Friedman ANOVA trend test indicated that mRNA-based vaccination induced a significant increase in median AAA1 IgG levels in both healthy controls and RA over time. In healthy controls, the median AAA1 IgG levels difference between 0.24 and 0.32 OD_{405nm} was significant ($p = 0.0001$) and the delta of 0.08 OD_{405nm} exceeded the LSC of 0.06 OD_{405nm}. In RA, the median difference between 0.27 and 0.30 OD_{405nm} was found to be marginally significant ($p = 0.04$), without exceeding the LSC (delta: 0.03 OD_{405nm}). However, in both populations, these medians trends did not translate into significant increases in AAA1 IgG seropositivity rates despite evolving from 5 to 10% in healthy controls and from 9 to 12.9% in RA patients (Table 1).

Finally, there were no associations between AAA1 IgG and symptoms of any kind during the vaccination protocol and no correlations between anti-S1 antibodies and AAA1 IgG responses could be identified at any time points (data not shown).

4 | DISCUSSION

The study results suggest that mRNA-based anti-SARS-CoV-2 vaccination induces a significant AAA1 IgG response in healthy controls and RA within the first two months after injection. Albeit significant at the median levels, such phenomenon did not exceed the LSC in RA patients and did not increase AAA-1 IgG seropositivity rates in both study groups. However, because raised AAA1 IgG levels have been shown to bear incremental prognostic information for incident cardiovascular events in different populations, including in RA,[9,13] the present biological signature identified in this underpowered and hypothesis-generating study should be further investigated, especially as the AAA1 IgG seropositivity rate doubled at 8 weeks after the second vaccination in healthy controls. Although preliminary and requiring further confirmation, these results are in line with the general established concept that exposure to pathogens epitopes through natural infections or vaccination may, in genetically predisposed individuals, lead to the development of autoimmunity.[1–3]

In the specific context of COVID-19 and AAA1 IgG, the present results indicate that if increased, the vaccination-induced AAA1 IgG response would still be by far less important to what has been shown with natural SARS-CoV-2 exposure where AAA1 IgG seropositivity rates exceeded 80% of patients within 10 days post-symptoms onset.[8] On a further reassuring note, the close associations previously reported between AAA1 IgG and anti-SARS-CoV-2 humoral responses[8] could not be reproduced in vaccinated individuals, and no associations were found between patients-reported symptoms and AAA1 IgG levels, in opposition to what has been observed in COVID-19 (manuscript under review). Although reassuring, the reasons for such differences are unclear and may indicate that other additional mechanisms (ie intra-intermolecular spreading) to molecular mimicry between self-antigens apoA-1 and Spike epitopes may be at stake to explain the COVID-19-induced AAA1 IgG response.

The AAA1 IgG response in humans has been shown to be preferentially oriented against the c-terminal (c-ter) part of apoA-1 (amino acids (aa): 216–243; reviewed in Ref. 9) which shares sequence homologies with c-ter of Spike (aa: 1139–1162).[8] Because this apoA-1 c-ter region plays a key role in the cellular cholesterol efflux and HDL development[14] and because of the linear inverse relationships reported between AAA1 IgG levels and HDL levels and their anti-oxidant functions (reviewed in Ref. 9), we expect the vaccine-induced anti-apoA-1 IgG response to be associated with lower HDL levels and loss of HDL anti-oxidant properties. However, such hypothesis is devoid of any experimental evidence and because the impact of vaccination on AAA1 IgG levels was presently much lower than the one induced

TABLE 1 Baseline characteristics of RA patients and healthy controls

	RA patients ($n = 77$)	Healthy controls ($n = 20$)	p
Age, mean (±SD)	63.6 (12.7)	44.8 (13.9)	<0.0001
Female gender, *n* (%)	46 (59.7)	15 (75)	0.45
Vaccination type/schedule			
mRNA-1273, *n* (%)	12 (15.6)	0 (0)	0.06
BNT162b2, *n* (%)	65 (84.4)	20 (100)	
Mean interval between 1st vaccination and sampling (days ± SD)	21.4 ± 2.3	21.8 ± 2	0.33
Mean interval between 2nd vaccination and sampling (days ± SD)	14.4 ± 2.6	15.2 ± 1.6	0.22
Mean interval between 1st and 2nd vaccination (days ± SD)	34.5 ± 4	32.9 ± 5.9	0.15
RA disease characteristics			
ACPA and/or RF positivity, *n* (%)	47/75 (62.7)	NA	
RA disease duration (years ± SD)	9.2 (8.7)	NA	
DMARD therapy			
csDMARDs monotherapy, *n* (%)	22 (28.6)	NA	
bDMARDs, *n* (%)	35 (45.5)	NA	
Monotherapy of bDMARDs, *n* (%)	14 (40)	NA	
JAK inhibitors, *n* (%)	20 (26)	NA	
Monotherapy of JAK inhibitors, *n* (%)	8 (20)	NA	
Prednisone, *n* (%)	25 (32.5)	NA	
Mean daily dose prednisone (mg ± SD)	5.6 ± 3.6	NA	
Serologies			
Baseline			
Median AAA1 levels (IQR)	0.27 (0.20–0.42)	0.24 (0.18–0.33)	0.39
AAA1 seropositivity, *n* (%)	7 (9.0)	1 (5.0)	1
Median anti-S1 levels, U/ml (IQR)	0.4 (0.4–0.4)	0.4 (0.4–0.4)	0.72
Anti-S1 seropositivity, *n* (%)	0 (0)	0 (0)	1
After 1st vaccination			
Median AAA1 levels (IQR)	0.30 (0.19–0.41)	0.27 (0.19–0.36)	0.68
AAA1 seropositivity, *n* (%)	9 (9.0)	1 (5.0)	1
Median anti-S1 levels, U/ml (IQR)	0.4 (0.4–6.0)	99.2 (24.8–172)	<0.0001
Anti-S1 seropositivity, *n* (%)	28 (36.3)	20 (100)	<0.0001
After 2nd vaccination			
Median AAA1 levels (IQR)	0.29 (0.18–0.39)	0.28 (0.21–0.41)	0.63
AAA1 seropositivity, *n* (%)	7 (9.0)	1 (5.0)	1
Median anti-S1 levels, U/ml (IQR)	687 (147–2500)	2500 (2500–2500)	<0.0001
Anti-S1 seropositivity, *n* (%)	69 (89.6)	20 (100)	0.20
8 weeks after 2nd vaccination			
Median AAA1 levels (IQR)	0.30 (0.19–0.46)	0.32 (0.24–0.42)	0.57
AAA1 seropositivity, *n* (%)	10 (12.9)	2 (10)	0.45
Symptoms during vaccination			
Any, *n* (%)	57 (74.05)	14 (70)	0.78
Systemic, *n* (%)	42 (54.5)	11 (55)	1
Local, *n* (%)	48 (62.3)	8 (40)	0.08

Note: All continuous variables are expressed as median (interquartile range, IQR; and range).

Abbreviations: ACPA, anti-citrullinated protein autoantibodies; RF, rheumatoid factor; DMARD, Disease-modifying antirheumatic drugs; Cs, conventional synthetic; B, biologic; JAK, Janus kinase; AAA1, anti-apolipoprotein A-1.

*p value derived from the comparison between RA and healthy control.

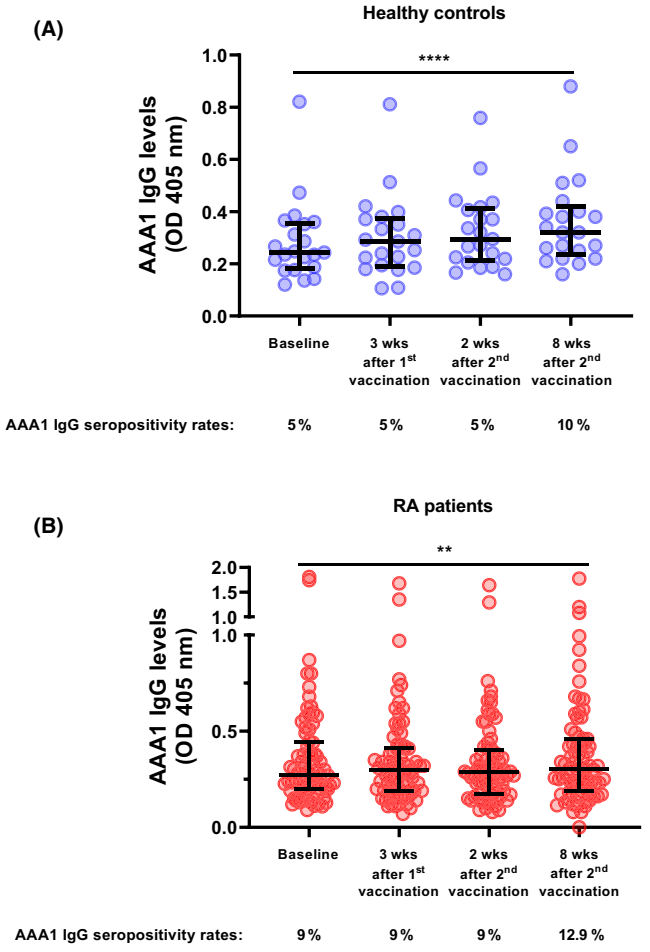

FIGURE 1 Evolution of AAA1 IgG levels up to 8 weeks after 2nd vaccination (±5 days) on 20 healthy controls and 77 RA patients. mRNA-based vaccination induced a significant increase in median AAA1 IgG levels over time in healthy controls, panel (A) as well as in RA patients, panel (B). Results are expressed as median with interquartile range and the Friedman trend test was used to compare the four groups. ****$p < 0.0001$ and **$p = 0.0043$. Samples were analysed in duplicate. Wks, weeks

by natural exposure to SARS-CoV-2,[8] we may at this stage only assume a minor and likely neutral clinical impact of anti-SARS-CoV-2 vaccination on HDL/apoA-1 levels and functions. On the other hand, as AAA1 IgG directly elicit the production of major pro-inflammatory cytokines such as interleukin (IL)-6, IL-8 and tumour necrosis factor-α (reviewed in Ref. 9) by interacting with the leucine-rich repeat region of TLR2 (aa 456–464) sharing sequence homologies with the aa region 455–487 of Spike,[8] we cannot rule out that the vaccination-induced AAA1 IgG response could in turn promote a low-grade pro-inflammatory state, paving the way for long-term and potentially pleiotropic complications. Such hypothesis-generating concept being properly addressable only by large multi-centric studies benefiting of several years of follow-up, we could not challenge this assumption in the present study.

Our exploratory study has additional limitations. The first relates to its very limited size and duration follow-up, raising obvious power concerns. In this context, retrieving significant results and observing a doubling of the seropositivity rate 24 weeks after the second injection should be interpreted as a biological signature of caution, until the formal demonstration of its harmlessness, especially because the detection of AAA1 IgG has been shown to precede the development of clinical manifestations by several years (reviewed in Refs. 9,13). Secondly and along the same line, this study was not designed to assess any potential clinical implications or to challenge the prognostic value usually ascribed to AAA1 IgG. Knowing whether a vaccine-induced AAA1 IgG response could have any clinical impact relating to the mRNA vaccine-induced myopericarditis[15] or to the CV prognostic implications of COVID-19[16] is unknown. Thirdly, as we purposely restricted our investigation on AAA1 IgG being previously shown to be raised by COVID-19[8] and to be of likely CV prognostic relevance (reviewed in Ref. 9), determining whether the present findings may apply to other autoantibodies of clinical relevance in COVID-19[4–6] needs to be established. Furthermore, AAA1 IgG levels in RA patients showed an important inter-individual variability for which the current study design does not allow us to identify an underyling cause. Moreover, we did not study the AAA1 IgG associations with lipid profile or HDL functions. Last but not least, we could not challenge the impact of other vaccines than mRNA-based ones on AAA1 IgG levels due to their unavailability in Switzerland. However, these results suggest that screening for autoimmune biological signatures in the context of emergent vaccines roll-out may provide complementary information to post-marketing surveillance programmes.

In conclusion, as opposed to what has been reported with COVID-19 infection, mRNA-based vaccination seems to induce a light AAA1 IgG response in immunocompetent individuals within 2 months after the last injection. Although we did not observe any warning signs, the formal demonstration of the harmlessness of such biological signature would be required.

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CONFLICTS OF INTEREST

AR-R reports consulting fees from AbbVie, Gilead, Lilly, BMS and Sanofi, honoraria from AbbVie, Pfizer, Sanofi, UCB, BMS, Lilly, Gilead and Roche, payment for expert testimony from AbbVie and Gilead, support for travel or meeting attendance from Sanofi, Roche, and AbbVie,

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