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Virus-specific antibody responses in severe acute respiratory syndrome coronavirus 2-infected and vaccinated individuals

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ABSTRACT

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection can have a serious course with many complications, especially in immunocompromised individuals. In such persons, other latent virus infections may contribute to disease pathology, in particular viruses which infect immune cells such as Epstein-Barr virus (EBV) and cytomegalovirus (CMV).
Methods: In this study, serology-based assays were conducted to analyse antibody responses to SARS-CoV-2 spike protein (SP), EBV Epstein-Barr nuclear antigen (EBNA)-1 and CMV phosphoprotein (pp)52 in naturally SARS-CoV-2-infected individuals, non-infected healthy controls (HCs) and vaccinated healthy controls (VHCs) to identify an association between SARS-CoV-2 antibodies and EBV and CMV antibodies in order to determine whether latent EBV and CMV infected individuals are more prone to become infected with SARS-CoV-2. Moreover, SARS-CoV-2, EBV, and CMV antibody responses were characterized in serum from patients with relapsing-remitting multiple sclerosis (RRMS), a chronic inflammatory disease strongly associated with EBV infections, to determine whether the serologic virus antibody profile varies in immunocompromised RRMS individuals upon SARS-CoV-2 vaccinations compared to VHCs.
Results: Significantly elevated SP IgG, IgM and IgA levels were identified in SARS-CoV-2-infected immunocompetent individuals when compared to non-infected HCs. However, no correlation was found to serum antibodies between SARS-CoV-2, EBV, and CMV in individuals infected with SARS-CoV-2 and in VHCs, suggesting that latent infections with neither EBV nor CMV associates to SARS-CoV-2 infection. Moreover, no significant difference in SP IgG, IgA and IgM levels was observed between vaccinated RRMS patients and VHCs, indicating that the immune system of immune deficient RRMS patients and VHCs respond identical to SARS-CoV-2 vaccinations.
Conclusion: Collectively, SARS-CoV-2 SP antibody levels reflect the vaccination and infection history and do not associate with EBV and CMV serostatus.

1. Introduction

One of the most critical functions of the immune system is to protect the host from infections. A well-functioning immune system efficiently neutralizes infections, whereas immuno-compromised individuals are more vulnerable to infections [21]. Whether resulting from impaired cellular functions, the absence of central proteins in immune regulation or the presence of autoreactive cells, immunocompromised individuals are more exposed to infections such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), cytomegalovirus (CMV) and Epstein-Barr virus (EBV), and may suffer from recurring infections [35].

SARS-CoV-2 is one of the recent viruses identified to cause a

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worldwide pandemic, coronavirus disease-2019 (COVID-19) [22]. As of June 2024, >775 million cases of COVID-19 counting >7 million deaths have been reported to the WHO (https://covid19.who.int/). SARS-CoV-2 is a single-stranded RNA virus, whose genome only encodes relatively few proteins [12]. Some of the important proteins include spike protein (SP), nucleocapsid, membrane and envelope proteins, which are essential for production of complete virus particles [64].

SARS-CoV-2 belongs to the coronavirus family, which causes illnesses ranging from common colds to more severe diseases such as Middle East respiratory syndrome coronavirus (MERS-CoV) [13,34,51,64]. SARS-CoV-2 typically induces respiratory infections, which can be asymptomatic or cause fever, cough, severe pneumonia, and in worst cases death [16]. Immunocompromised individuals have been reported to have a more severe disease course and a poorer outcome compared to healthy individuals [28]. Especially individuals with hematologic malignancies have been reported to have an increased risk for associated morbidity and mortality, which limit prevention, treatment and clearance of the SARS-CoV-2 virus [28]. Based on this, SARS-CoV-2 has been extensively studied due to the potential threat on society [28,31,59].

SARS-CoV-2 infections were originally described as highly contagious and rapidly spreading, resulting in a high infection rate [7,52,63]. Similar to SARS-CoV-2, EBV infections are associated with a high infection rate, as EBV infects at least 95 % of the adult population [32,43,50]. EBV infections are associated with severe diseases such as various carcinomas and various chronic immune defective diseases such as multiple sclerosis (MS) [6,9,19,20,32,33,49,57,62]. Although presenting with a high infection rate, EBV infections are often not discovered in immunocompetent individuals and most EBV infections remain subclinical [53]. When infected with EBV during or after adolescence, EBV commonly causes infectious mononucleosis, which among others increases the risk of developing MS [25,39,61]. Upon initial infection, EBV persists for lifetime by remaining latent in among others memory B cells [2,18,30,36,46,56,68]. After primary infection is resolved and latency is established, antibodies to the latent EBV nuclear antigen-1 (EBNA-1) are produced at high levels, indicating a persistent, yet subclinical latent infection [11,56]. Occasionally, EBV reactivates and enters more active latent and lytic states. Healthy individuals are mostly asymptomatic to reactivations, whereas reactivation in immunocompromised individuals may have fatal outcomes [46,68].

Similar to EBV, Cytomegalovirus (CMV) is a human herpes virus that is endemic throughout the world with rates reaching 100 % in some developing countries [27,40]. Primary CMV infection is usually asymptomatic in immunocompetent individuals, although nearly 10 % of infected individuals present with symptoms similar to the self-limiting mononucleosis-like syndrome [40,41,58]. CMV infections may cause substantial morbidity and mortality in immunocompromised individuals or patients suffering from hematological disorders [26]. Similar to EBV, CMV remains latent in the host upon the acute phase of initial infection and is thought to reactivate, leading to a smoldering latent infection [3].

Virologic relations to EBV and CMV can be difficult to determine, as individuals infected with SARS-CoV-2 typically are infected with EBV and/or CMV prior to SARS-CoV-2 infection. It has been indicated that human herpes viruses reactivate during SARS-COV-2 infection, however it is unclear whether reactivation of CMV and/or EBV infections occurs in the context of a severe SARS-CoV-2 infection or whether smoldering latent infections influence the pathogenesis of SARS-CoV-2 infections [1,24,69].

In this study we describe serologic virus antibody levels in SARS-CoV-2 infected individuals, healthy controls (HCs), vaccinated healthy controls (VHCs) and immunocompromised relapsing-remitting multiple sclerosis (RRMS) patients, to determine a possible serologic correlation between SARS-CoV-2, CMV and EBV antibodies.

2. Materials and methods

2.1. Materials

Alkaline phosphatase (AP)-conjugated anti-human IgG/IgA/IgM, Tris base, Tris HCL and *para*-nitrophenylphosphate (*p*NPP) substrate tablets were from Sigma (St. Louis, Mo, USA). NaCl was purchased from Unikem (Copenhagen, Denmark). Diethanolamine, Tween 20, Na_2CO_3, $NaHCO_3$, phenol red and $MgCl_2$ were from Merck (Darmstade, Germany). The ectodomain of SP from SARS-CoV-2 was from Baltymas (Vilnius, Lithuania) and was produced in-house at the institute of Biotechnology, Vilnius University, Lithuania. CMV pp52 was from Prospec Protein Specialists (Ness-Ziona, Israel). Full-length EBV EBNA1 was from MyBiosource (San Diego, CA, USA). PolySorb microtiter plates were from Thermo Fisher, NUNC (Roskilde, Denmark).

2.2. Human patient samples

Serum samples were collected from SARS-CoV2-infected individuals (n = 96) and HCs (n = 100), which represented Caucasians of both genders and all ages (Table 1). Samples were collected from SARS-CoV-2-positive individuals visiting a clinical physician between April and December in 2020. The majority of the enrolled SARS-CoV-2 infected individuals experienced relative mild disease symptoms and only a limited number of the included individuals required additional care for recovery. SARS-CoV-2-positive serum samples were collected at the Department of Clinical Immunology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark and were used anonymously. Samples were tested positive for SARS-CoV-2 by PCR prior to enrollment.

Samples from HCs were from the Danish Blood Bank at Statens Serum Institut, Copenhagen, Denmark. HCs samples were negative for any known diseases and were used anonymously. HCs were negative for both SARS-CoV-2 infection and vaccination. HCs were enrolled prior to the SARS-CoV-2 pandemic.

Serum samples from vaccinated HCs (VHCs) (n = 15) and RRMS patients (n = 13) were collected at the department of Neurology, Rigshospitalet Glostrup, Denmark, which was approved by the Regional Scientific Committee of Copenhagen (no H-19036891). RRMS patients enrolled were newly diagnosed and had not received any immune modulating treatments. All VHCs and RRMS patients had been vaccinated three times in average with a 50:50 ratio of Moderna and Pfizer-BioNTech vaccines. None of the RRMS patients had been naturally infected with SARS-CoV-2 prior to vaccination. Vaccinated individuals were recruited from May 2021 to June 2023.

None of the RRMS patients enrolled received any MS-related therapy at the time of sample collection to ensure that serum antibody levels were not influenced by treatment strategy, as some disease-modifying treatments may reduce vaccine responses [10,17]. Signed informed consent was obtained from all participants.

Table 1
Patient characteristics. Serum from four cohorts were included: fully vaccinated healthy controls (VHCs), patients with severe acute respiratory syndrome-corona virus-2 (SARS-CoV-2) infection, non-infected healthy controls (HCs) and fully vaccinated relapsing-remitting multiple sclerosis (RRMS) patients.

	SARS-CoV-2-infected individuals	VHC	Vaccinated RRMS patients	HCs
n	96	15	13	100
Gender (M:F)	-	5:10	4:9	50:50
Average age (years)	-	43.19	40.54	41.32
Age range (years)	-	26–68	18–66	19–65

2.3. Detection of virus antibodies in serum samples

Virus antibody levels were quantified by enzyme-linked immunosorbent assay as previously described [29,38]. Briefly, ninety-six-well PolySorb microtiter plates were coated with virus antigen (EBV EBNA1, CMV pp52, SARS-CoV2 SP) (1 μg/mL) diluted in carbonate buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, 0.5 % Phenol red, pH 9.6) and incubated over night at room temperature (RT). Unbound protein was removed by washing the wells with Tris-Tween-NaCl (TTN) buffer (50 mM Tris, 1 % Tween 20, 0.3 M NaCl, pH 7.5) (200 μL) for 3 × 1 min. Following rinsing, the wells were further blocked by addition of TTN to all wells and incubation at RT for 30 min on a shaking table. TTN buffer was removed, followed by addition of human sera diluted in TTN (1:100) (100 μL) and the plate incubated at RT with gentle shaking. After 1 hour of incubation, excess serum was removed and the wells were rinsed with TTN for 3 × 1 min, goat anti-human IgG-AP(/IgM-AP/IgA-AP) was added to all wells (1 μg/mL) and incubated as previously described. Unbound antibody-enzyme conjugates were removed by washing the wells with TTN for 3 × 1 min (200 μL), whereafter AP-substrate buffer (1 mg/mL *p*NPP in 1 M diethanolamine, 0.5 mM $MgCl_2$, pH 9.8) was added to all wells. Bound antibodies were quantified by measuring absorbances at 405 nm using a microplate reader (Molecular Devices, Menlo Park, CA, USA). Corrected absorbances were obtained by subtracting the background signal for each sample from its respective absorbance at 650 nm.

For calculation of SP IgG, EBNA1 IgG and CMV IgG concentrations a standard curve (U/mL) was generated by using two folds serial dilution of a positive serum pool, starting from a 1:100 dilution. A pool of anti-SP-positive samples diluted in TTN (1:100) was used as a high positive control. All samples were tested in duplicates and the mean of blank replicates was subtracted from the measured absorbances before final antibody concentrations.

2.4. Data analysis

Myassays.com was used to generate a 4-parametric logistic curve fit, where initial absorbances corrected for background noise was used to calculate IgG levels (U/mL) in the individual samples.

Statistical analyses and visualization of results obtained were conducted using GraphPad Prism software (v 5, Graphpad, San Diego, CA, USA). Pearsons's correlation analyses were determined, where r was defined as follows: 0–0.25 = no correlation, 0.25–0.5 = weak positive correlation, 0.5–0.75 = moderate positive correlation, 0.75–1.0 = strong positive correlation. Statistical significance was assessed by nonparametric unpaired two-tailed Mann Whitney tests. Significant difference is indicated by: *: $p< 0.05$, **: $p< 0.01$, ***: $p< 0.001$.

3. Results

3.1. Screening of patient sera for antibody reactivity to severe acute respiratory syndrome-coronavirus-2 spike protein

Initially, preliminary screenings were conducted to determine total SP Ig levels in serum pools from SARS-CoV-2 infected individuals, HCs, VHCs, and RRMS patients. Elevated IgG levels were observed in SARS-CoV-2-infected and VHC pools, whereas reduced IgM and IgA levels were observed when compared to IgG levels. SARS-CoV-2 SP IgG, IgM, and IgA levels were notably elevated in infected individuals compared to HCs, whereas SP IgA and IgG levels were reduced when compared to VHCs and RRMS patient pools (Fig. 1). In contrast, SARS-CoV-2-infected individuals presented with elevated IgM levels when compared to the vaccinated pools, VHCs and RRMS patients. Both VHCs and RRMS pools obtained similar SP IgG and IgA levels, whereas elevated SP IgM levels were found for the VHC pool when compared to the RRMS pool. Individual SARS-CoV-2-infected serum samples presented with significantly elevated SP IgG, IgM and IgA levels compared to HCs ($p< 0.0001$) (Fig. 2). Moreover, all SP antibody isotypes examined effectively separated SARS-CoV-2 infected individuals from non-infected HCs, as illustrated by AUC scores ranging between 0.8167 (SP IgA) to 0.9970 (SP IgG) ($p< 0.0001$).

High sensitivities were obtained for SP IgA and SP IgM, depending on the defined specificity. Presenting with a specificity of 95 %, sensitivities of 56–57 % were obtained, whereas sensitivities of 68–82 % were obtained with a specificity of 80 %. In contrast, SP IgG yielded a sensitivity of 99 % and a specificity of 100 %, clearly illustrating that S IgG almost perfectly separates SARS-CoV-2-infected individuals from non-infected individuals (Fig. 2, Table 2).

Thorough characterization of serologic SP antibody levels in naturally infected individuals demonstrated that 99, 73nd 55 % of serum samples were positive for IgG, IgM and IgA, respectively (Fig. 3). In total, 31 % of samples were positive for all three isotypes, whereas 20 % only expressed IgG and IgM. Thirty-five % of infected samples only presented with one isotype, of these 34 % were only positive for IgG and finally 1 % was weakly positive for IgM.

Further characterization revealed that patient samples positive for all three isotypes (IgG+ IgA+ IgM+) in general presented with the highest individual isotype responses when compared to the remaining groups. This observation was however only statistically significant for SP IgG levels ($p= 0.0125$ for IgG+ IgA+ IgM-) ($p< 0.0001$ for IgG+ IgA- IgM+ ($p= 0.0002$ for IgG+ IgA- IgM-) and SP IgM level when compared to the IgG+ IgA- IgM+ group ($p= 0.0274$). No significant difference in SP IgA levels was observed between the IgA positive groups ($p= 0.1246$ for IgG+ IgA+ IgM-) examined in this cohort.

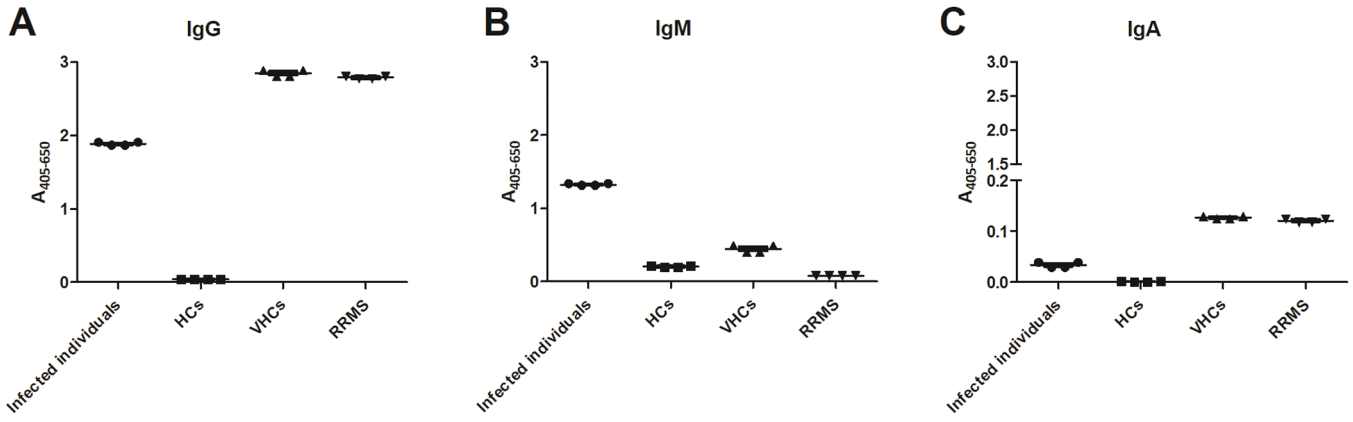

Fig. 1. Antibody pool reactivity to spike protein in severe acute respiratory syndrome coronavirus-2-infected individuals, healthy controls, vaccinated healthy controls and relapsing-remitting multiple sclerosis patients analysed by enzyme-linked immunosorbent assay. (*A)* SP IgG reactivity in serum pools. (B) SP IgM reactivity in serum pools. (C) SP IgA reactivity in serum pools.

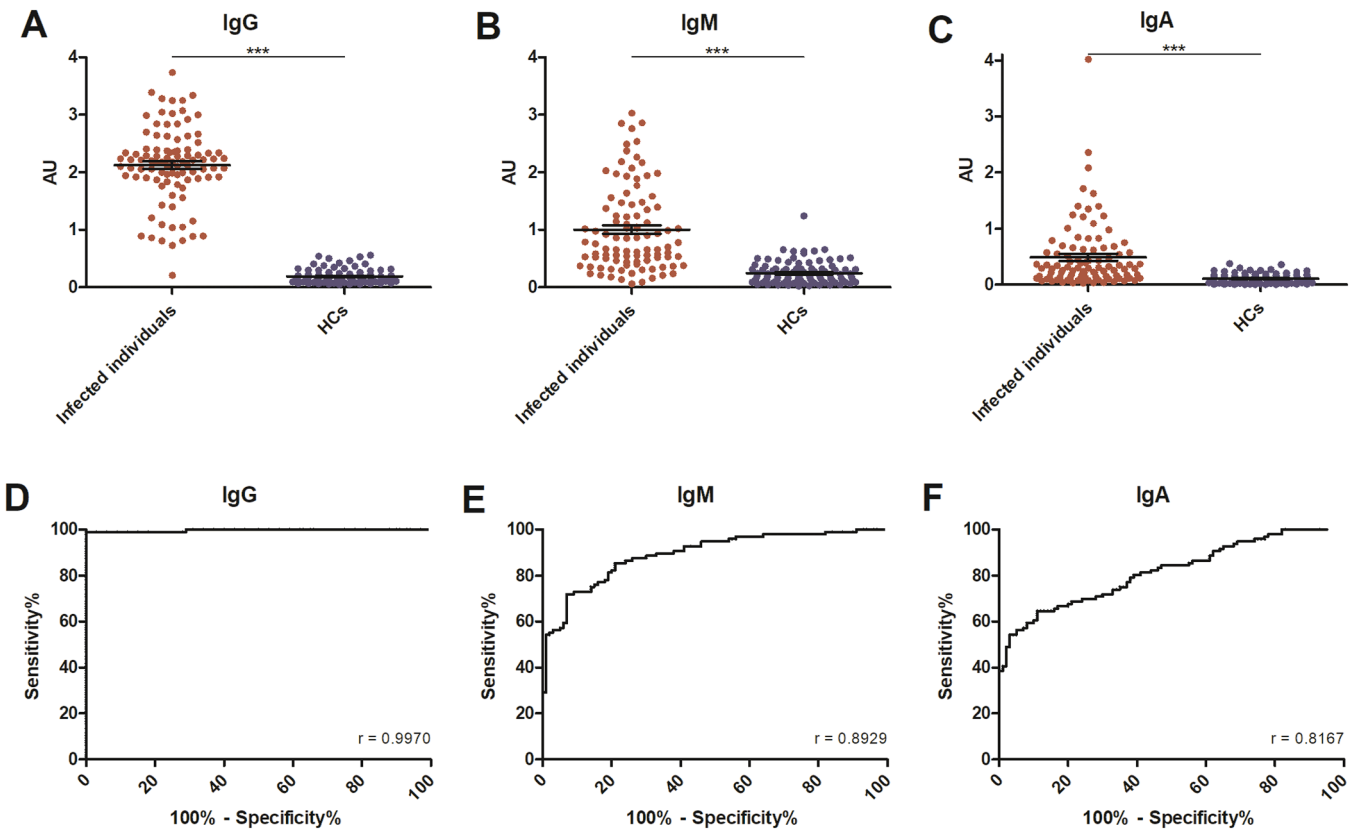

Fig. 2. Spike protein IgG, IgM and IgA in severe acute respiratory syndrome-coronavirus-2-individuals and non-infected healthy controls analysed by enzyme-linked immunosorbent assay. (*A)* SPIgG reactivity. (B) SP IgM reactivity. (C) SP IgA reactivity. (D) ROC curve for SP IgG in infected individuals and HCs. (E). ROC curve for SP IgM in infected individuals and HCs. (F). ROC curve for SP IgA in infected individuals and HCs.

Table 2
Spike protein IgA, IgG and IgM reactivities in severe acute respiratory syndrome-coronavirus-2 positive individuals and non-infected healthy controls. SP IgA and S IgM sensitivities were determined based on 80 % (*) and 95 % (**) specificities.

	SARS-CoV-2 infected individuals			Non-infected healthy controls		
	IgG	IgA	IgM	IgG	IgA	IgM
n	96			100		
Mean	2.12	0.49	1.00	0.18	0.11	0.24
Range	0.20–3.73	0.03–4.01	0.06–2.85	0.05–0.55	0.01–0.37	0.02–0.65
Sensitivity	99	68*(56**)	82*(57**)	-	-	-
Specificity	100	80(95)	80(95)	-	-	-

3.2. Screening for spike protein IgG, IgM and IgA in SARS-CoV-2 vaccinated individuals

Following screening of serum samples from naturally SARS-CoV-2-infected individuals, serum from VHCs and vaccinated RRMS patients were analysed for SP Ig reactivity and compared to non-infected HCs (Fig. 4). Serum samples from RRMS patients and VHCs presented with elevated SP IgG, IgM and IgA titers when compared to HCs ($p< 0.0001$). No significant difference in SP IgG ($p= 0.3568$), IgM ($p = 0.3814$) and IgA ($p = 0.4610$) levels was determined between RRMS patients and VHCs samples tested, indicating that the immune system of VHCs and vaccinated RRMS patients respond equally to SARS-CoV-2 vaccination. Analysis of isotype profiles found that 73 % and 93 % of VHCs and RRMS samples were positive for both all three isotypes (IgG, IgM and IgA), respectively. Collectively, SP antibody profiles in VHCs and RRMS patients at first glance appeared to differ when compared to naturally SARS-CoV-2 infected individuals.

3.3. Antibody correlation in serum of relapsing-remitting multiple sclerosis patients, infected individuals and vaccinated healthy controls

To thoroughly characterize the humoral antibody response to SP in these individuals, correlations between SP IgG, IgA and IgM were determined.

As presented, significant correlations were identified between SP antibody isotypes SARS-CoV-2-infected individuals (Fig. 5). Weak positive correlations were evident for SP IgM and IgG ($r= 0.2073$, $p= 0.0427$) and for IgA and IgG ($r= 0.3175$, $p= 0.0016$), whereas a moderate positive correlation was identified between SP IgA and IgM ($r= 0.4753$, $p< 0.0001$). In contrast, no significant correlations could be determined for SP antibody levels detected in samples from VHCs ($p> 0.05$), although a weak positive non-significant correlation was determined between SP IgA and IgG levels ($r= 0.2386$, $p= 0.3918$). Similarly, weak positive correlations were identified for IgG and IgA ($r= 0.2224$, $p= 0.4652$) and IgA and IgM ($r= 0.4878$, $p= 0.0908$) in vaccinated RRMS patients, although not statistically significant.

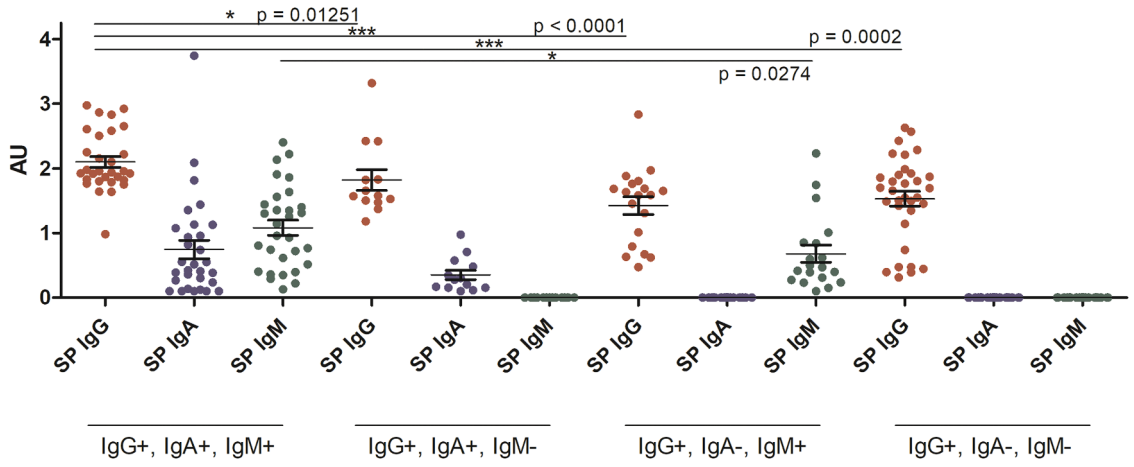

Fig. 3. Isotype distribution spike protein antibodies in SARS-CoV-2 infected individuals analysed by enzyme-linked immunosorbent assay. Samples were identified as positive by subtracting the GNS+2SD for the HCs for all respective antibody isotypes. A. Venn diagram illustrating SP IgG, IgM and IgA isotype distribution in SARS-CoV-2-infected individuals. B. Individual SP Ig levels based on distribution profile.

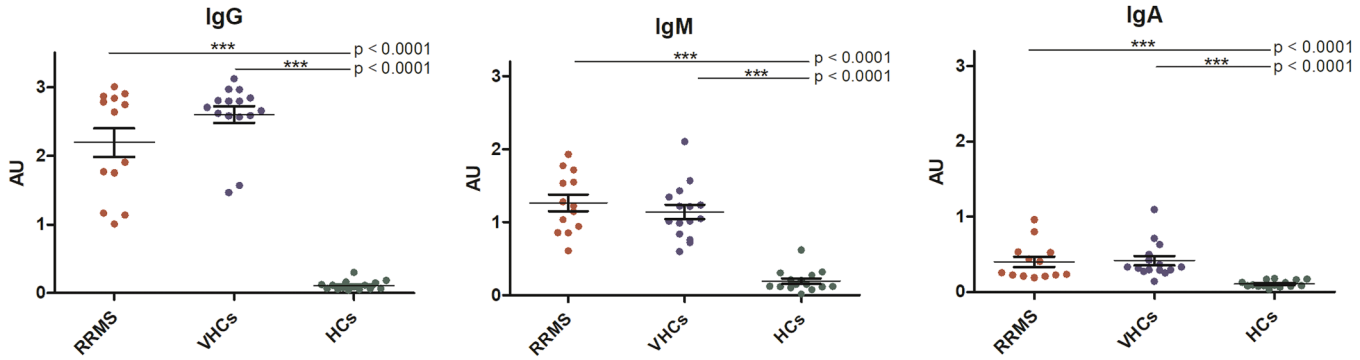

Fig. 4. Reactivity of sera from relapsing-remitting multiple sclerosis patient, vaccinated healthy controls and non-infected healthy controls to spike protein. Samples were identified as positive by subtracting the GNS+2SD for the HCs for all respective antibody isotypes. *(A)* SP IgG reactivity. (B). SP IgM reactivity. (C). SP IgA reactivity. (D). Isotype distribution in RRMS patients. (E) Isotype distribution in VHCs.

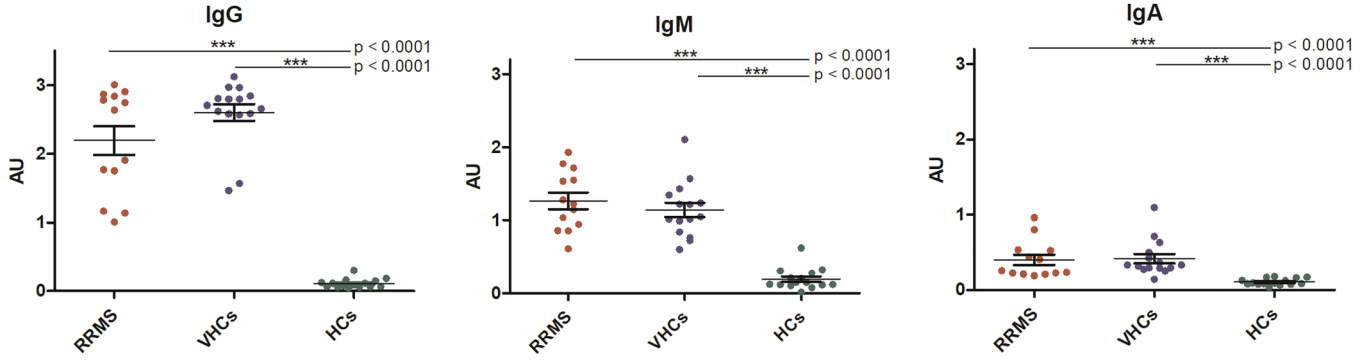

Fig. 5. Antibody correlations between spike protein antibody isotypes IgG, IgA and IgM in SARS-CoV-2-infected individuals (A-C), vaccinated healthy controls (D-F) and vaccinated relapsing-remitting multiple sclerosis patients (G-I).

3.4. EBV EBNA1 and CMV pp52 virus antibody levels in SARS-CoV-2 associated samples

To investigate serologic correlations between SARS-CoV-2 and some of the most common viruses, EBV and CMV, antibody levels to EBV EBNA1 and CMV pp52 were determined in SARS-CoV-2-infected individuals, VHCs and RRMS patient samples and compared to serum SP IgG levels.

Fig. 6A illustrates antibody reactivity to SARS-CoV-2 SP, EBV EBNA1 and CMV pp52 in SARS-CoV2-infected individuals. EBV EBNA1 IgG and CMV pp52 IgG were significantly lower when compared to SP IgG levels ($p< 0.0001$). Moreover, CMV pp52 IgG levels were significantly elevated when compared to EBNA1 IgG ($p= 0.0003$). In contrast, a potential trend indicated elevated EBNA1 IgG levels in VHCs ($p= 0.0564$) and RRMS patients ($p= 0.0727$) compared to SP IgG levels. No difference in CMV pp52 IgG and SP IgG levels were observed for VHCs ($p= 0.9669$) and RRMS patients ($p= 0.3560$) in this patient population. A potential trend indicated elevated EBNA1 IgG titers in serum from RRMS patients when

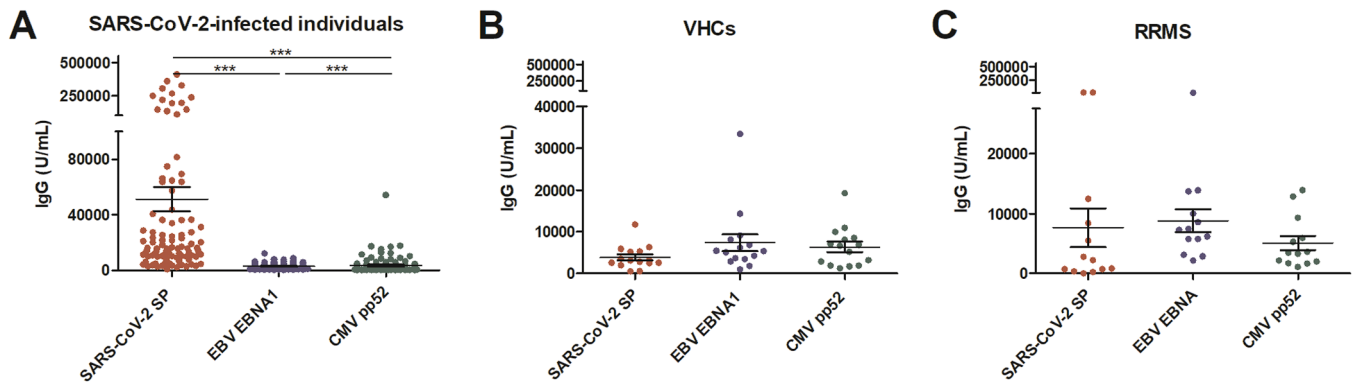

Fig. 6. SARS-CoV2 Spike protein IgG, EBV EBNA1 IgG and CMV pp52 IgG in serum of SARS-CoV-2-infected individuals, vaccinated healthy controls and vaccinated relapsing-remitting multiple sclerosis patients. (A) Virus IgG levels in SARS-CoV-2-infected individuals. (B). Virus IgG levels in SARS-CoV-2 vaccinated healthy controls. (C). Virus IgG levels in serum of SARS-CoV2-vaccinated relapsing-remitting multiple sclerosis patients.

compared to CMV pp52 IgG (p= 0.0727), which is in accordance with the profound role of EBV in the development of MS [9,33,67].

Following evaluation of virus IgG levels, correlations between SP IgG, SP IgA and SP IgM and EBV EBNA1 IgG and CMV pp52 IgG were determined (Fig. 7, Table 3). As illustrated, no correlations in this cohort were identified between SP IgG, IgM and IgA to neither EBNA-1 IgG nor CMV pp52 IgG in SARS-CoV2-infected individuals. Moreover, no notable correlation was determined between CMV pp52 and EBV EBNA1 IgG levels when compared to SARS-CoV-2 SP IgG, IgA and IgM (Table 3) in VHCs and RRMS patient samples analyzed.

Collectively, based on serologic studies the current findings do not indicate that serum SP Ig levels in SARS-CoV-2-infected individuals correlate with neither CMV or EBV IgG. These findings may indicate that immune responses to natural infections and vaccine antigens differ in response and composition, which even may be compromised due to the

Table 3
Correlations between SARS-CoV-2 Ig and EBV EBNA1 IgG and CMV pp52 in SARS-CoV-2-infected individuals, vaccinated healthy controls and relapsing-remitting multiple sclerosis patients.

	SP IgG	SP IgM	SP IgA
SARS-CoV2-infected individuals			
EBV EBNA-1	*−0.0471*	*0.1539*	*0.0753*
CMV pp52	*0.1828*	*0.0705*	*0.1664*
Vaccinated healthy controls			
EBV EBNA-1	*−0.2707*	*0.0872*	*−0.1537*
CMV pp52	*0.0510*	*- 0.0012*	*−0.0670*
Multiple sclerosis patients			
EBV EBNA-1	*0. 0598*	*0.2303*	*−0.1729*
CMV pp52	*−0.1618*	*0.2048*	*−0.2457*

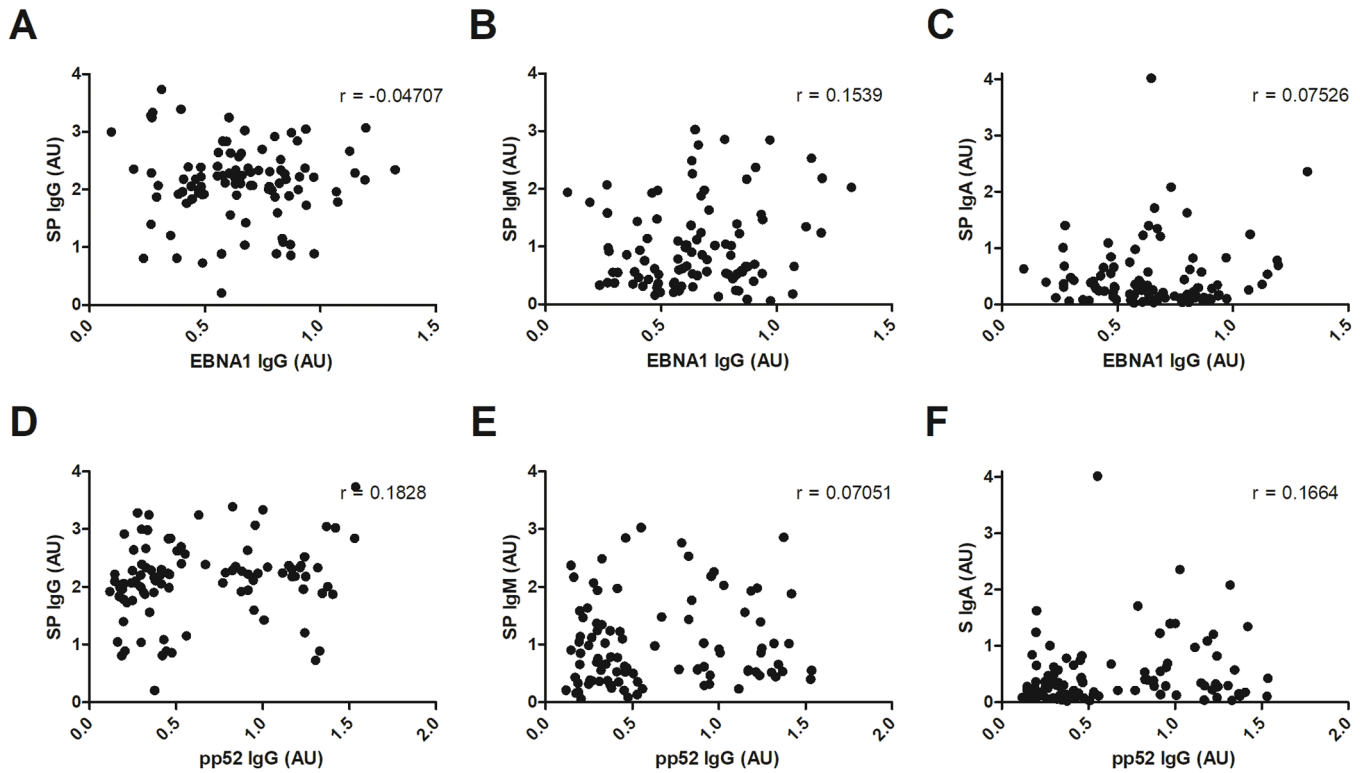

Fig. 7. Correlation between EBV EBNA1 IgG, CMV pp52 and SP IgG, IgM and IgA in SARS-CoV-2-infected individuals. A. Correlation between SP IgG and EBNA1 IgG. B. Correlation between SP IgM and EBNA1 IgG. C. Correlation between SP IgA and EBNA1 IgG. D. Correlation between SP IgG and pp52 IgG. E. Correlation between SP IgM and pp52 IgG. F. Correlation between SP IgA and CMV pp52 IgG.

immune status of the infected and/or vaccinated individuals. This remains to be elaborated.

4. Discussion

In this study, serologic antibody levels associated to SARS-CoV-2, EBV and CMV infections were investigated. Most naturally, preliminary pool screenings found that SP IgG levels were significantly elevated in naturally infected SARS-CoV-2 individuals compared to non-infected HCs, whereas SP IgG and IgA levels were elevated in vaccinated individuals when compared to infected SARS-CoV-2 and HCs, suggesting that antibody responses to vaccines yield high antibody titers compared to natural infection, which is in accordance with the literature [5,54,65]. Lower antibody responses in naturally infected individuals with mild symptoms compared to those with more severe disease courses have been reported, which conforms to that the majority of the enrolled individuals experienced relative mild disease courses and only a limited number of the included SARS-CoV-2 positive individuals required hospitalization [4,14,47].

Following pool screenings, individual patient samples were screened for SP IgA, IgM and IgG reactivity to determine the serologic humoral immune response to SP. Results obtained demonstrated distinct differences in antibody profiles following natural SARS-CoV-2 infection. Ninety-nine % of the naturally infected HCs were positive for SP IgG, whereas approximately 53 % of the serum samples were positive for all three isotypes. These findings are in contrast to isotype distributions obtained for the vaccinated RRMS patients and VHCs, describing that close to 100 % of all samples were positive for all 3 antibody isotypes. The presence of SP antibody profiles has previously been analysed with respect to disease severity, but no correlation between the presence of isotype-specific SARS-CoV-2 antibodies and the disease course could be determined in this cohort, [42].

The difference in antibody isotype distribution may be ascribed the difference in immune responses generated as a response to a natural infection when compared to a vaccine-generated immune response. These findings are supported by that antibody isotype distribution correlations between the three groups were different, as the highest correlations were determined for the infected HCs, whereas no notable correlations were observed for VHCs and vaccinated patients with RRMS. Collectively, these main findings appear to be associated with differences in the infection/vaccination status, rather than a poor humoral immune response in immunocompromised RRMS patients, as RRMS and VHCs present with similar antibody levels and distribution profiles compared to naturally infected individuals.

These observations are in accordance with recent studies describing the frequency of COVID-19 in immunocompromised individuals, showing that these individuals do not experience increased susceptibility to SARS-CoV-2 infection nor experience more severe forms of COVID-19 [37,45,48].

No serologic correlation was observed between SP IgG and CMV pp52 IgG or EBV EBNA1 IgG in the examined cohort, suggesting SARS-CoV-2 infection is not associated with CMV or EBV coinfection or/and that coinfection not necessarily leads to a more severe disease outcomes, which previously has been suggested [15,55]. SARS-CoV-2 infection has been suggested to reactivate EBV, as studies describe that SARS-CoV-2 infection reactivates EBV at a higher rate than in non-infected individuals [8]. In this process, the presence of SARS-COV-2 antigens from an inflammatory stimulus may lead to terminal differentiation and activation of latent EBV in B cells, allowing transition from the latent to the lytic phase of the virus [15,55]. Although no correlation between EBV and SARS-CoV-2 could be determined at the serologic level, SARS-CoV-2 infection may lead to reactivation of EBV, which has been suggested to result in more severe SARS-CoV-2 disease courses [1,24].

A few reports on SARS-CoV-2 and CMV co-infection have been described [44,60,66,69]. Previous findings reported that CMV seropositivity was associated with more than twice the risk of hospitalization due to SARS-CoV-2 infection and that CMV seroprevalence was higher for hospitalized SARS-CoV-2 infected individuals [69]. CMV replication predominates in the lungs, a major reservoir for CMV, and local reactivation may cause lung injury and/or result in complications associated with CMV and critical illness [23]. Thus, CMV infection has the potential to shape the course of SARS-CoV-2 infections, which typically target the upper respiratory tract, either because of CMV reactivation or due to the broader reshaping of cytotoxic lymphocyte populations [69]. Furthermore, it has been suggested that CMV-seropositive individuals may have a higher baseline level of innate immune activation, leading to a greater inflammatory response and increased disease severity during SARS-CoV-2 infection [44].

Collectively, SARS-CoV-2 SP antibody levels reflect the vaccination and infection history of the individuals and does not depend on the EBV or CMV serostatus for infection.

CRediT authorship contribution statement

Nicole Hartwig Trier: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nadia Zivlaei:** Writing – review & editing, Investigation. **Sisse Rye Ostrowski:** Writing – review & editing, Resources. **Erik Sørensen:** Writing – review & editing, Resources. **Margit Larsen:** Writing – review & editing, Resources. **Rimantas Slibinskas:** Writing – review & editing, Resources. **Evaldas Ciplys:** Writing – review & editing, Resources. **Jette Lautrup Frederiksen:** . **Gunnar Houen:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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