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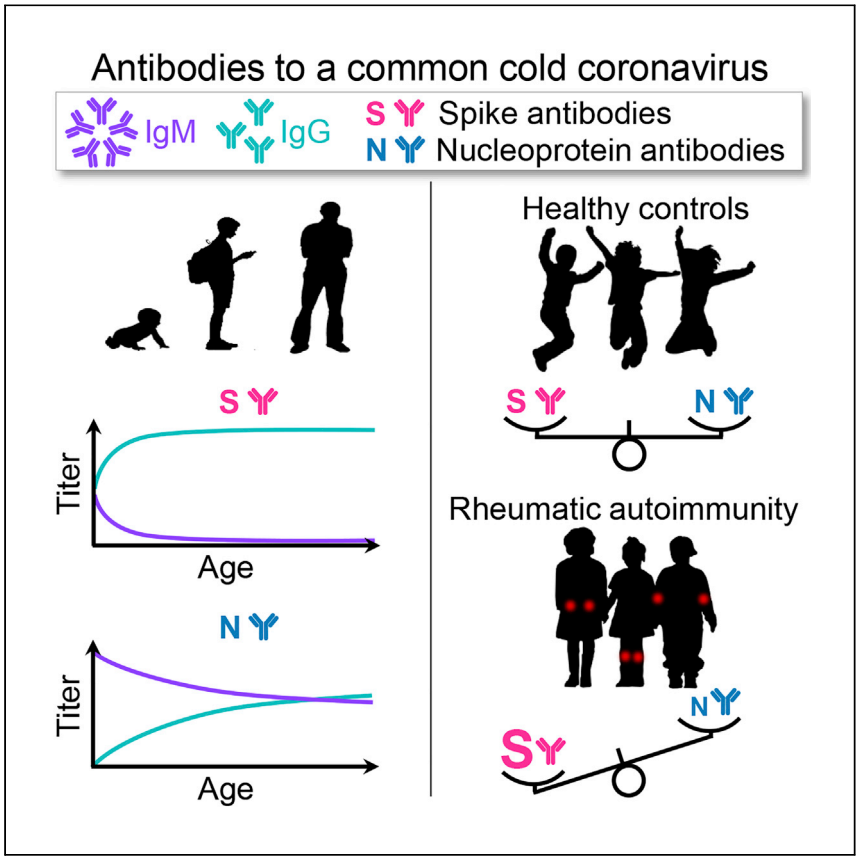
Favorable antibody responses to human coronaviruses in children and adolescents with autoimmune rheumatic diseases



Deakin et al. examined the antibody response to the common-cold coronavirus HCoV-OC43 and cross-reactive response to SARS-CoV-2 in pre-COVID-19 pandemic sera from JIA, JDM, and JSLE patients. They found that these prevalent inflammatory rheumatic diseases or their immunosuppressive treatment did not adversely affect the response to a common-cold coronavirus.

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Highlights

IgG dominates the antibody responses to HCoV-OC43 spike in children and adolescents

Response to the HCoV-OC43 nucleoprotein exhibits delayed age-dependent class-switching

Higher response to HCoV-OC43 spike but not nucleoprotein in JIA, JDM, and JSLE patients

Pre-COVID-19 pandemic JIA, JDM, and JSLE patient sera cross-react with SARS-CoV-2 spike

Translation to Patients

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Clinical and Translational Article

Favorable antibody responses to human coronaviruses in children and adolescents with autoimmune rheumatic diseases

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SUMMARY

Background: Differences in humoral immunity to coronaviruses, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), between children and adults remain unexplained, and the effect of underlying immune dysfunction or suppression is unknown. Here, we sought to examine the antibody immune competence of children and adolescents with prevalent inflammatory rheumatic diseases, juvenile idiopathic arthritis (JIA), juvenile dermatomyositis (JDM), and juvenile systemic lupus erythematosus (JSLE) against the seasonal human coronavirus (HCoV)-OC43 that frequently infects this age group.

Methods: Sera were collected from JIA (n = 118), JDM (n = 49), and JSLE (n = 30) patients and from healthy control (n = 54) children and adolescents prior to the coronavirus disease 19 (COVID-19) pandemic. We used sensitive flow-cytometry-based assays to determine titers of antibodies that reacted with the spike and nucleoprotein of HCoV-OC43 and cross-reacted with the spike and nucleoprotein of SARS-CoV-2, and we compared them with respective titers in sera from patients with multisystem inflammatory syndrome in children and adolescents (MIS-C).

Findings: Despite immune dysfunction and immunosuppressive treatment, JIA, JDM, and JSLE patients maintained comparable or stronger humoral responses than healthier peers, which was dominated by immunoglobulin G (IgG) antibodies to HCoV-OC43 spike, and harbored IgG antibodies that cross-reacted with SARS-CoV-2 spike. In contrast, responses to HCoV-OC43 and SARS-CoV-2 nucleoproteins exhibited delayed age-dependent class-switching and were not elevated in JIA, JDM, and JSLE patients, which argues against increased exposure.

Conclusions: Consequently, autoimmune rheumatic diseases and their treatment were associated with a favorable ratio of spike to nucleoprotein antibodies.

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Context and significance

Children and adolescents with rheumatic diseases are considered to be at risk of COVID-19. However, data on the ability of such patients to fight SARS-CoV-2 are lacking, as infections are rare due to shielding or undetected due to a lack of severe symptoms and limited mass testing. Instead, Deakin et al. studied how well such patients defended themselves against a common-cold coronavirus, HCoV-OC43, a relative of SARS-CoV-2 that frequently infects this age group. By studying children and adolescents with arthritis, dermatomyositis, or lupus before the COVID-19 pandemic, they found that these prevalent inflammatory rheumatic diseases did not impede the antibody response to a common-cold coronavirus, raising the possibility that the response to SARS-CoV-2 may also be unaffected.

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INTRODUCTION

Four types of human coronaviruses (HCoVs) are endemic in the human population, causing frequent infection with relatively mild disease.[1–4] The multiple introductions of zoonotic coronaviruses in the last couple of decades have highlighted their considerable pathogenic potential.[5] This is exemplified by the pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that currently continues to spread globally.[6]

The outcome of SARS-CoV-2 infection is highly variable both in presentation and prevalence. Several subtypes of coronavirus disease 19 (COVID-19) are now recognized, ranging from a mild flu-like, severe gastrointestinal or respiratory disease to multiorgan failure.[6,7] Severe complications of COVID-19 are comparatively rare and depend on age, sex, ethnicity, access to healthcare, socioeconomic status, and underlying health conditions.[6,7] A sizable proportion of SARS-CoV-2 infections may also be asymptomatic, particularly in younger individuals.[8] Children appear relatively protected from severe COVID-19.[9] This observation mirrors findings from previous epidemics caused by SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV), again sparing children, although transmission of earlier zoonotic coronaviruses was much less widespread or documented.[10] In contrast, children experience more frequent infections than adults with one or more of the four seasonal HCoVs[3,11] and are more likely to harbor pre-existing antibodies and memory B cells that cross-react with SARS-CoV-2.[12,13]

Although they appear protected from severe COVID-19, unexplained inflammation following SARS-CoV-2 infection is being increasingly recognized in a very small fraction of children and adolescents.[14] The causes of this condition, termed pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 infection (PIMS-TS) or multisystem inflammatory syndrome in children and adolescents (MIS-C), remain unknown. A dysregulated antibody response has been proposed as one possible contributor,[15] highlighting the need for a better understanding of the immune response to SARS-CoV-2.

Management of the pandemic has necessitated public health measures, including shielding, aiming to protect vulnerable populations. An initial assessment in adult patients suggested an increased risk of COVID-19 associated with rheumatic and other autoimmune diseases, attributable to their immunosuppressive treatment.[16,17] Children and adolescents with the most prevalent inflammatory rheumatic diseases, such juvenile idiopathic arthritis (JIA), juvenile dermatomyositis (JDM), and juvenile systemic lupus erythematosus (JSLE) were initially considered to be potentially at risk, owing to humoral immune dysfunction and associated immunosuppressive treatments. However, data on the ability of such patients to mount a humoral response to coronavirus infections are lacking.

SARS-CoV-2 infections are relatively rare in children and adolescents with rheumatic diseases due to shielding and are relatively undetected in healthier children and adolescents due to a lack of severe symptoms and limited mass testing. Therefore, we studied antibody responses to one of the seasonal HCoVs, HCoV-OC43, which causes very frequent infections in this age group,[3,11] is the most prevalent of the four HCoVs in the United Kingdom,[18,19] and may also elicit antibodies that

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cross-react with SARS-CoV-2,[12,20] as a surrogate of antibody response to coronaviruses in general. We compared the antibody responses of pediatric and adolescent JIA, JDM, and JSLE patients to two immunodominant coronaviral antigens with those of their healthier peers. Our findings suggest that inflammatory rheumatic diseases do not impede humoral immunity to the seasonal HCoV-OC43 in this age group and may even enhance it.

RESULTS

Normal or stronger immunoglobulin G (IgG) responses to coronaviral spikes in pediatric and adolescent JIA, JDM, and JSLE patients

To detect and quantify antibodies reactive with HCoV-OC43 and SARS-CoV-2 spikes, we used a flow-cytometry-based assay, which relies on expression of the full-length spikes on the surface of HEK293T cells (Figure S1). Using this assay, we previously described the presence of SARS-CoV-2 spike-reactive antibodies in pre-COVID-19 sera collected from children and adolescents, as well as from a smaller fraction of adults.[12] To allow quantitation of relative antibody titers, we modified the assay by mixing equal ratios of HEK293T cells transfected to express each coronaviral spike (HEK293T.spike) with HEK293T cells transduced to express an unrelated retroviral envelope glycoprotein (HEK293T.env), which served as control, and GFP (Figure S1). Cells expressing HCoV-OC43 spike were mixed with cells expressing the human endogenous retrovirus (HERV) ERV3-1 envelope glycoprotein, and cells expressing SARS-CoV-2 spike were mixed with cells expressing HERV-K113 envelope glycoprotein. The specific increase in staining intensity was calculated by comparing the mean fluorescence intensity (MFI) of HEK293T.spike cells and HEK293T.env with the MFI of unstained cells.

In all healthy control and patient groups of children and young people (Table S1), IgG was the main class of spike-binding antibodies, representing 68% and 61% of all antibodies to HCoV-OC43 and SARS-CoV-2 spikes, respectively (Figure 1A). IgM antibodies represented 27% and 30% of antibodies to HCoV-OC43 and SARS-CoV-2 spikes, respectively, which was likely an overestimate, owing to the higher non-specific staining of IgM antibodies. In contrast, IgA antibodies were the least abundant, likely due to competition with IgG antibodies in the assay, as previously observed.[12]

Consistent with our previous findings,[12] the majority pre-pandemic serum samples from healthy children and adolescents had detectable IgG antibodies to HCoV-OC43 spike, and a smaller fraction had detectable antibodies to SARS-CoV-2 spike and at much lower levels (Figure 1A; Table 1). Pediatric and adolescent JIA, JDM, and JSLE patients showed a similar profile, with most having IgG antibodies to HCoV-OC43 spike and a fraction having IgG antibodies to SARS-CoV-2 spike (Figure 1A; Table 1). The prevalence of IgG antibodies to HCoV-OC43 spike was significantly higher in JSLE patients than that in healthy controls, as was the prevalence of IgG antibodies to SARS-CoV-2 spike (Table 1). As a positive control for SARS-CoV-2-infection-induced antibodies, we included a small group of patients with MIS-C. As expected,[21] levels of antibodies to SARS-CoV-2 spike were substantially higher in MIS-C patients than those in other groups by a factor of over 20, whereas antibodies to HCoV-OC43 spike were comparable (Figure 1A).

Antibodies to ERV3-1 or HERV-K113 envelopes were very rarely detected in any group, with the exception of JSLE, which included several adolescent patients with high IgG antibody levels to ERV3-1, of which two also had detectable antibodies to HERV-K113, and of MIS-C, of which most patients had elevated levels of antibodies to HERV-K113 (Figure 1B).

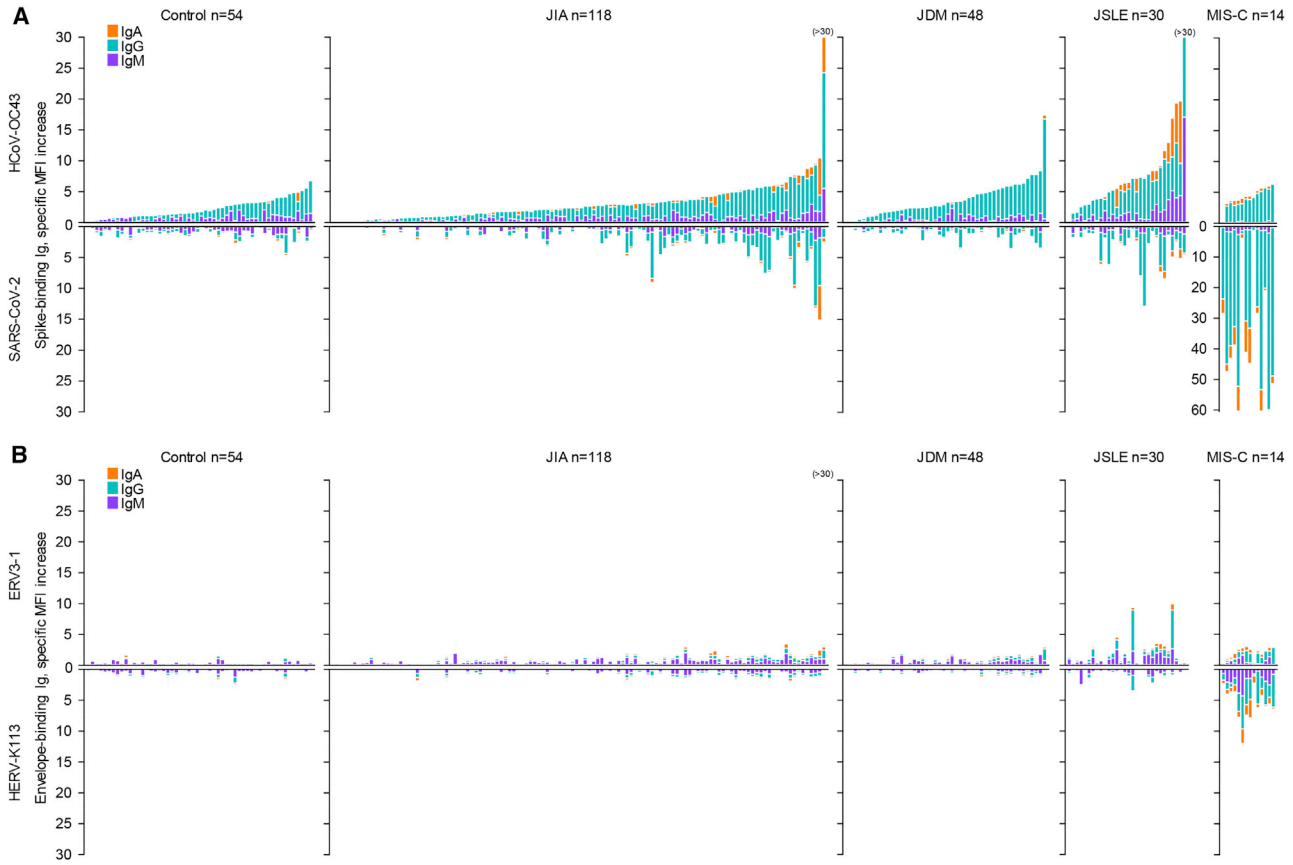

Figure 1. Antibodies to coronaviral spikes in pediatric and adolescent JIA, JDM, JSLE, and MIS-C patients and age-matched controls

(A) Mirror plots of the specific MFI increase of HEK293T cells expressing HCoV-OC43 spike (top) or SARS-CoV-2 spike (bottom) caused by individual sera. Each bar is an individual healthy control or patient. Samples are plotted according to the signal of antibodies to HCoV-OC43 spike and in the same position in the mirror plots. Antibody levels to SARS-CoV-2 spike in MIS-C patients are plotted on a different scale from the rest.

(B) Mirror plots of the specific MFI increase of HEK293T cells expressing ERV3-1 (top) or HERV-K113 (bottom) envelope glycoproteins caused by individual sera. Samples are plotted in the same order as in (A).

Given that adolescent JSLE patients were, on average, older than other patients and healthy controls (Table S1) and that age may influence antibody levels and class, we stratified each condition by age. We considered two age groups, namely, 1–12 and 13–18 years of age, referred to here as younger and older, respectively. Compared with the respective healthy controls, younger JIA and JDM patients had significantly higher levels of IgG antibodies to HCoV-OC43 spike, which were largely normalized in older JIA and JDM patients (Figure 2A). Significantly higher levels of IgG antibodies to HCoV-OC43 spike were also observed in JSLE patients (Figure 2A). As IgG antibodies were the predominant class, these differences were also reflected in the total antibodies to HCoV-OC43 spike (Figure 2A). IgA antibodies to HCoV-OC43 spike were also elevated in JSLE and older JIA patients (Figure 2A). Despite the fact that antibodies to SARS-CoV-2 spike were found at much lower levels and correlated weakly with antibodies to HCoV-OC43 spike (correlation coefficient 0.376 for IgG antibodies, $p < 0.0001$), their prevalence in pediatric and adolescent JIA, JDM, and JSLE patients mirrored antibodies to HCoV-OC43 spike (Figure 2B). Significantly higher levels of IgG antibodies to SARS-CoV-2 spike were observed in JSLE and younger JIA patients, whereas total antibodies to SARS-CoV-2 spike seemed to drop below control levels in older JIA patients (Figure 2B). The MIS-C group, which included only one patient over the age of 13, exhibited significantly higher levels of antibodies to HCoV-OC43 spike,

Table 1. Prevalence of IgG antibodies to OC43 and SARS-CoV-2 spikes in JIA, JDM, and JSLE patients.

	Prevalence of IgG to OC43 spike		Prevalence of IgG to SARS-CoV-2 spike	
Disease group	Positive/total (%)	p value[a]	Positive/total (%)	p value[a]
Control	40/54 (74.1)		21/54 (38.9)	
JIA	100/118 (84.7)	non-significant	57/118 (48.3)	non-significant
JDM[b]	44/48 (91.7)	non-significant	23/48 (47.9)	non-significant
JSLE	29/30 (96.7)	0.0447	25/30 (83.3)	0.0003

[a]Bonferroni-corrected p values from Fisher's exact tests between each disease group and the healthy control.
[b]Values were not available for one of the JDM patients.

as well as to SARS-CoV-2 spike, when younger MIS-C patients were compared with age-matched controls (Figures 2C and 2D). Five MIS-C patients had received intravenous immunoglobulin (IVIG) therapy prior to sampling that could have affected their HCoV-OC43 spike antibodies titers. However, these titers were not significantly different between MIS-C patients with prior IVIG therapy and those without ($p = 0.072$, two-tailed Student's t test).

In addition to the effect of the disease group, a multiple regression analysis suggested an independent effect of multiple other factors, including donor or patient sex, age, year of sampling, immunosuppressive treatment, and autoantibody presence. After controlling for the effects of female sex, older age, year of sample collection, and steroid treatment, total antibodies to the HCoV-OC43 spike were significantly higher in JSLE patients than those in the controls, whereas IgG antibodies to HCoV-OC43 spike were elevated in pediatric and adolescent patients of all three conditions (Figures S2A and S2B; Table S2). Female sex and steroid treatment at the time of sampling were associated with higher antibody levels. Although disease duration of less than 24 months correlated with higher antibody titers for HCoV-OC43 spike in univariate analyses, this was largely accounted for by increased representation of JSLE and JDM patients in this category (Figures S2C and S2D). JDM was associated with lower levels of total antibodies to SARS-CoV-2 spike, whereas JSLE was associated with higher levels of IgG antibodies to the SARS-CoV-2 spike, after the effects of female sex, older age, year of sample collection, and autoantibody presence were controlled for. Total and IgG antibodies to SARS-CoV-2 spike were elevated in younger patients and in those with autoantibodies (Figures S2A and S2B; Table S3).

As these rheumatic diseases affect more females than males, the former are overrepresented in the JIA, JDM, and JSLE groups (Table S1). Nevertheless, when only younger male patients were compared with younger male healthy controls, levels of IgG antibodies to HCoV-OC43 spike were still significantly higher in JIA and JDM ($p = 0.00000993$ and $p = 0.000728$, respectively; Student's t test), demonstrating that this effect is independent of patient sex.

The effect of the year of sampling may reflect the variation in HCoV-OC43 prevalence between winters in the United Kingdom,[19] affecting the strength of the contemporaneous antibody response. Alternatively, it may reflect antigenic drift in HCoV-OC43 over long periods of time, leading to escape from antibody response, as recently suggested for HCoV-229E.[22] The HCoV-OC43 spike sequence we used in this study was from a clone isolated in 2017, and it was, therefore, possible that it was better recognized by more recent than older sera.[22] However, no linear relationship was observed between titers of antibodies to HCoV-OC43 spike and sampling

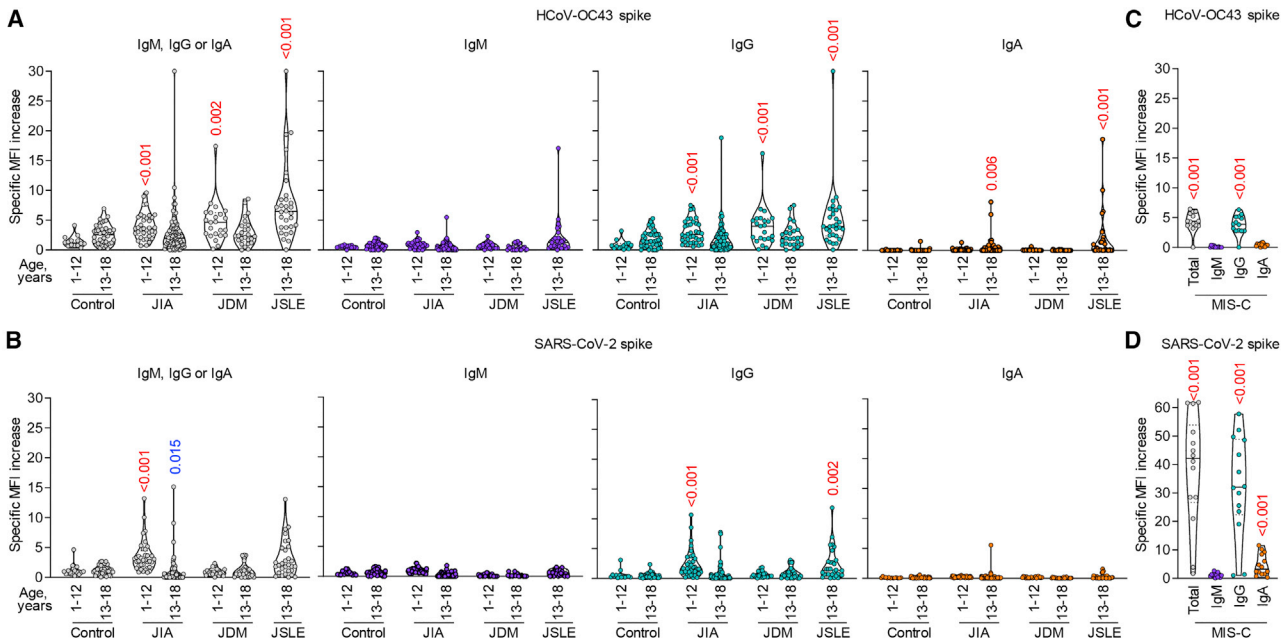

Figure 2. Antibodies to coronaviral spikes in pediatric and adolescent JIA, JDM, JSLE, and MIS-C patients and controls of different age

Specific MFI increases of HEK293T cells expressing HCoV-OC43 spike (A and C) or SARS-CoV-2 spike (B and D) caused by individual sera from the indicated age and disease group. Each symbol is an individual healthy control or patient. Antibody levels to SARS-CoV-2 spike in MIS-C patients are plotted on a different scale from the rest. Red and blue numbers within the plots denote the p values of statistically significant increases and decreases, respectively, when comparing each disease group with the respective healthy control of the same age group. The older control group was also compared with the younger control group.

year (Figure S3), arguing against the possibility that the 2017 HCoV-OC43 spike was not recognized by older sera. A similar pattern was seen also for antibodies that cross-reacted with SARS-CoV-2 spike (Figure S3), which are likely targeting more conserved regions of the spike and would not be affected by escape mutations in HCoV-OC43 spike.

The positive association between immunosuppressive steroid treatment and antibodies to coronaviral spike proteins was unexpected and remained even when other factors were taken into account (Figure S2; Table S2). Although it may not simply be a surrogate for disease activity, which necessitates higher doses, steroid treatment may still capture other underlying aspects of immune dysregulation, particularly in the JSLE group.

The positive correlation between antibodies to HCoV-OC43 and SARS-CoV-2 spikes and autoantibodies (e.g., antinuclear antibodies detected as part of diagnosis) was additionally supported by a similar correlation also with antibodies to HERV-encoded envelope glycoproteins, which are effectively autoantigens. Indeed, we observed a correlation between IgG to HCoV-OC43 or SARS-CoV-2 spikes and IgG autoantibodies to ERV3-1 (correlation coefficients of 0.692 [$p < 0.0001$] and 0.457 [$p < 0.0001$], respectively) or IgG autoantibodies to HERVK-113 (correlation coefficients of 0.277 [$p < 0.0001$] and 0.681 [$p < 0.0001$], respectively), indicating a common underlying cause, such as a hyperactive adaptive immune system.

To extend these observations, we used ELISAs to detect cross-reactive antibodies to SARS-CoV-2, as well as antibodies to other respiratory viruses. Using a recombinant SARS-CoV-2 S2 subunit fragment ($S_{686-1211}$), we detected cross-reactive IgG antibodies in most of the pre-pandemic samples and at similar levels between the

groups, with the exception of older JDM patients who had higher levels than their age-matched controls (Figure S4A). However, several studies noted disparities in the antibodies detected by ELISA by using stabilized recombinant monomeric fragments and antibodies detected by the trimeric wild-type SARS-CoV-2 spike or other methods,[12,23–26] confounding direct comparisons.

IgG antibodies to influenza A (IAV) H1N1 hemagglutinin (HA) were also detected in nearly all donors and patients and were generally higher in older than in younger individuals, with the exception of the JDM group ($p = 0.019$, when comparing all younger with all older individuals, except JDM patients; Mann-Whitney rank-sum test) (Figure S4B). Compared with their age-matched controls, JDM and JSLE patients exhibited significantly higher titers of IgG antibodies to IAV HA (Figure S4B). In contrast, levels of IgG antibodies to respiratory syncytial virus (RSV) fusion (F) protein were lower in older than those in younger individuals ($p < 0.001$, when comparing all younger with all older individuals; Mann-Whitney rank-sum test) and were also lower in older JDM and JSLE patients than in age-matched controls (Figure S4C). Thus, these results highlight virus-specific patterns, even among viruses with seemingly overlapping age-related prevalence.

To further examine the quality of antibodies to the intact, wild-type HCoV-OC43 and SARS-CoV-2 spikes detected by flow cytometry, we measured the kinetics of dissociation after spike binding, which is considered a surrogate of antibody affinity.[27] To this end, we engineered SUP-T1 cells to stably express SARS-CoV-2 spike and GFP (SUP-T1.spike cells) and multiplexed them with negative SUP-T1 cells, which served as an internal control for the staining intensity (Figure S5). This analysis indicated a potentially lower affinity of SARS-CoV-2 S cross-reactive IgG antibodies in JIA patients than that in controls but comparable affinity in JDM and JSLE patients (Figure S6). Antibody affinities determined by this method were lower in pre-pandemic samples than those in MIS-C patients and also lower than the affinity of the SARS-CoV-2 S2-reactive D001 monoclonal antibody (Figure S6), but differences were not significant with the small number of samples analyzed.

As an independent method of antibody detection and also of potential functional relevance, we assessed the ability of sera to neutralize SARS-CoV-2 *in vitro*. Most, but not all, of the sera with SARS-CoV-2 S-cross-reactive IgG, detected by flow cytometry, also exhibited *in vitro* SARS-CoV-2 neutralizing activity (Figure 3A). SARS-CoV-2 neutralization titers were similar between the three conditions and between age groups and comparable with those previously reported in healthy children and adolescents, but they were considerably lower than those detected in MIS-C patients (Figure 3A), in keeping with titers of binding antibodies. Neutralization activity showed a weak correlation with IgG to SARS-CoV-2 spike, which was expected given that not all spike-binding antibodies are neutralizing, but no correlation was found with IgG to HCoV-OC43 spike (Figure 3B).

Collectively, these data suggest that pediatric and adolescent JIA, JDM, and JSLE patients mounted a comparable, if not stronger, class-switched response to HCoV-OC43 spike, compared with healthy children and adolescents and harbored cross-reactive antibodies to SARS-CoV-2 spike.

Predominant IgM responses to coronaviral nucleoproteins in children and adolescents

Stronger IgG responses to coronaviral spikes in pediatric and adolescent JIA, JDM, and JSLE patients than those in healthy age-matched controls could potentially arise

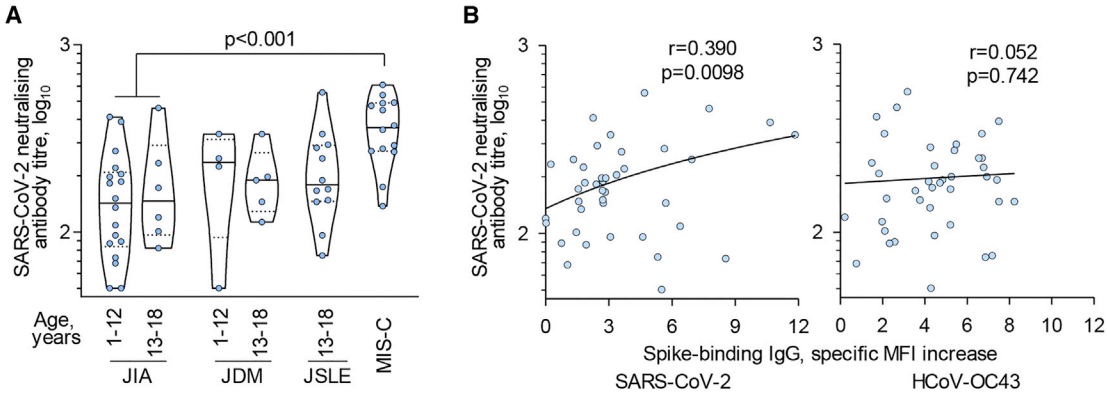

Figure 3. SARS-CoV-2 neutralizing antibodies in pediatric and adolescent JIA, JDM, JSLE, and MIS-C patients

(A) SARS-CoV-2-neutralizing antibody titers in the indicated age and disease group. Only patients with SARS-CoV-2 spike-binding antibodies detectable by flow cytometry were included. JIA, JDM, and JSLE patients of both age groups combined were compared with MIS-C patients by ANOVA on ranks tests.

(B) Correlation of SARS-CoV-2-neutralizing antibody titers with levels of flow-cytometry-detectable antibodies to SARS-CoV-2 spike (left) or HCoV-OC43 spike (right). In (A) and (B), each symbol is an individual patient. In (B), one JSLE patient was removed from the regression analysis as an outlier for the HCoV-OC43 spike antibodies, based on the Kurtosis coefficient of the group.

from immune hyperactivity to comparable exposure to HCoVs, as may be expected from the underlying rheumatic disease. Alternatively, it could arise from higher exposure to or infection with HCoVs in these patients, potentially due to immunosuppressive treatments. To explore these two possibilities, we assessed responses to a second coronaviral antigen, the nucleoprotein. As their target is an internal viral protein, antibodies to nucleoprotein are not neutralizing and their levels are thought to better reflect the amount of viral replication than antibodies to spikes.

To quantify antibodies to HCoV-OC43 and SARS-CoV-2 nucleoproteins, we developed a quantitative bead-based assay (Figure S7). Using this assay, we were able to detect HCoV-OC43 nucleoprotein-binding antibodies in effectively all samples (99.6%) and SARS-CoV-2 nucleoprotein-binding antibodies in a substantial proportion (70.1%) (Figure 4A). Surprisingly, however, the dominant class of nucleoprotein-binding antibodies in these cohorts was IgM, making up to 82.2% and 67.3% of antibodies to HCoV-OC43 and SARS-CoV-2 nucleoproteins, respectively. In contrast, antibodies to the HCoV-OC43 nucleoprotein in MIS-C patients were predominantly IgG, and expectedly,[21] antibodies to the SARS-CoV-2 nucleoprotein were elevated and also almost exclusively IgG (Figure 4B).

The dominance of IgM antibodies to nucleoproteins contrasted with the dominance of IgG antibodies to spikes in children found here and in prior reports[28] and also with reports of class use in antibodies to antigens from other viruses in children versus adults in general.[13] We therefore compared samples from healthy donors across the age spectrum to establish whether the apparent dominance of IgM antibodies to nucleoproteins was due to the assay used or indeed a unique feature of the response of children and adolescents to these nucleoproteins in particular. Healthy adults responded to the HCoV-OC43 nucleoprotein with a balanced IgG and IgM response, comprising 31.4% and 61.9% of the total response to this antigen (Figure 4C). This response of adults was significantly skewed compared with that of healthy children and adolescents, which comprised 8.7% IgG and 84.6% IgM ($p < 0.001$ when comparing the IgG or IgM titers to HCoV-OC43 nucleoprotein between healthy children/adolescents and healthy adults; Mann-Whitney U test). Thus, children and adolescents made an antibody response to coronaviral nucleoproteins

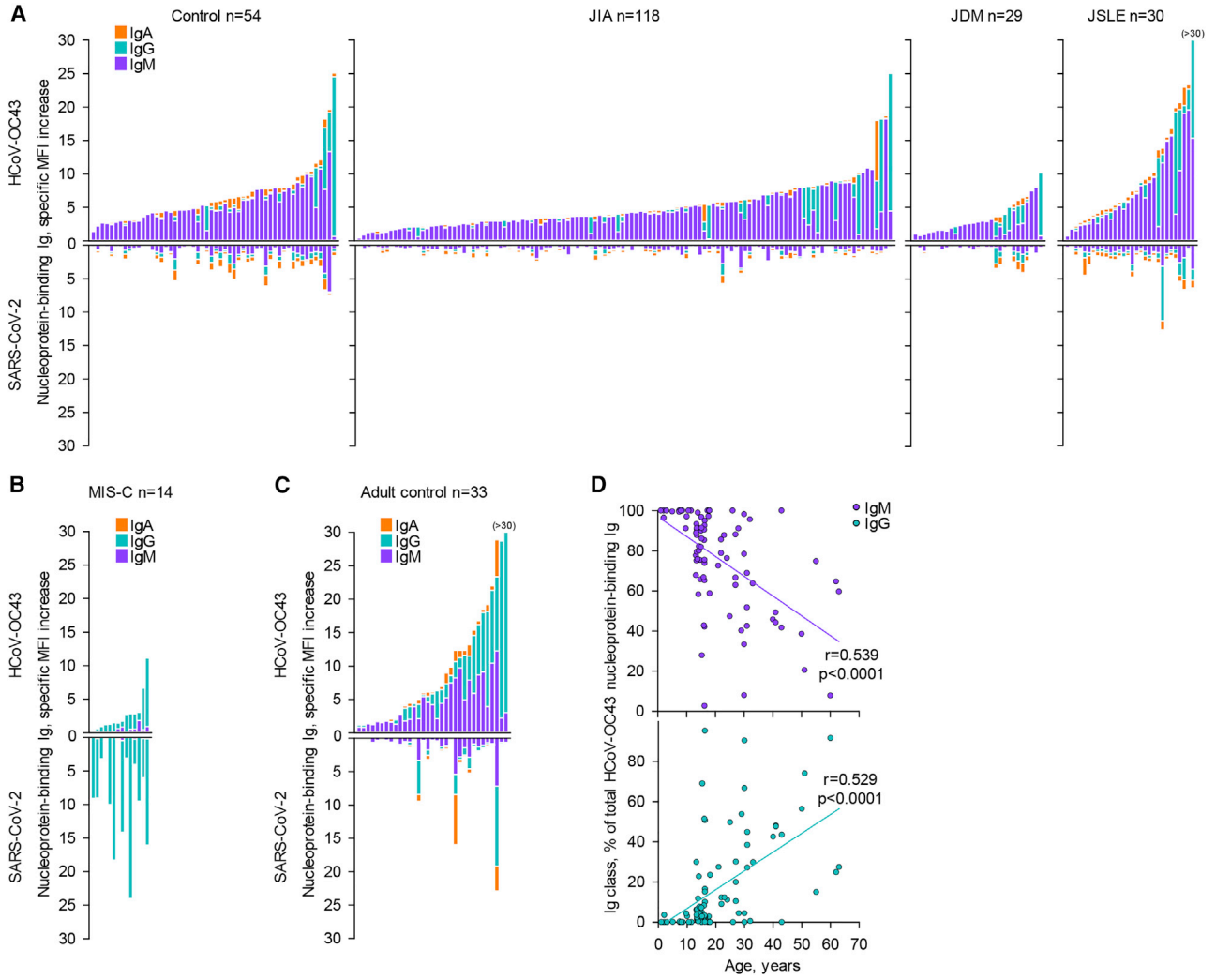

Figure 4. Antibodies to coronaviral nucleoproteins in pediatric and adolescent JIA, JDM, JSLE, and MIS-C patients and age-matched controls

(A–C) Mirror plots of the specific MFI increase of HEK293T cells expressing HCoV-OC43 nucleoprotein (top) or SARS-CoV-2 nucleoprotein (bottom) caused by individual sera. Each bar is an individual child or adolescent healthy control or JIA, JDM, or JSLE patient (A), MIS-C patient (B), or adult healthy control (C). Samples are plotted according to the signal of antibodies to the HCoV-OC43 nucleoprotein and in the same position in the mirror plots. (D) Correlation of the proportion of total HCoV-OC43 nucleoprotein-binding antibodies represented by IgM (top) or IgG (bottom) classes, and the age of the donor or patient. Each symbol is an individual sample.

that was unusually dominated by IgM and that gradually and partially switched to IgG with age (Figure 4D).

In comparison with the respective healthy control group, pediatric and adolescent JIA, JDM, and JSLE patients showed no evidence for elevated antibody responses to the HCoV-OC43 nucleoprotein (Figure 5A). These responses were, in fact, reduced in certain groups, such as older JIA and younger JDM patients (Figure 5A). A similar profile was observed also for antibodies to the SARS-CoV-2 nucleoprotein, with a significant increase with age in the healthy control group, and decrease in older JIA patients compared with their healthy control (Figure 5B). Multiple regression models confirmed that total antibodies and IgM antibodies to both HCoV-OC43 and SARS-CoV-2 nucleoproteins were reduced in JDM and JIA patients, after controlling for the effects of female sex and older age (Figure S2; Tables

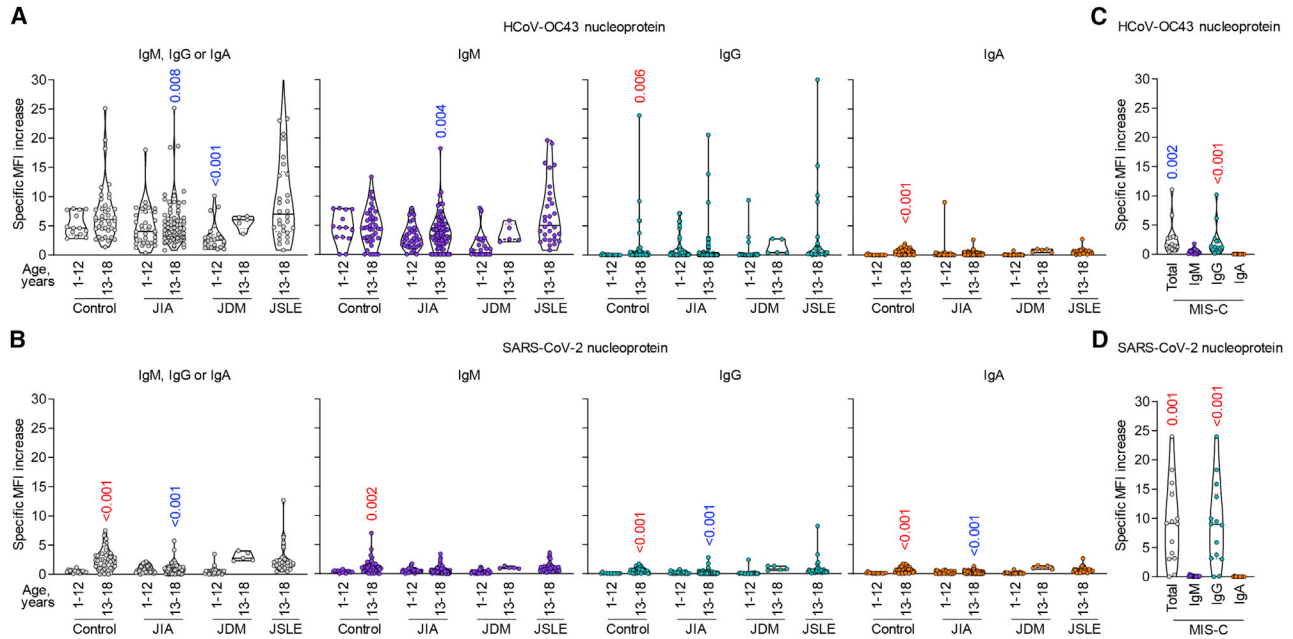

Figure 5. Antibodies to coronaviral nucleoproteins in pediatric and adolescent JIA, JDM, JSLE, and MIS-C patients and controls of different ages

Specific MFI increase of HEK293T cells expressing HCoV-OC43 nucleoprotein (A and C) or SARS-CoV-2 nucleoprotein (B and D) caused by individual sera from the indicated age and disease group. Each symbol is an individual healthy control or patient. Red and blue numbers within the plots denote the p values of statistically significant increases and decreases, respectively, when comparing each disease group with the respective healthy control of the same age group. The older control group was also compared with the younger control group.

S4 and S5). By comparison, titers of total antibodies to the HCoV-OC43 nucleoprotein were reduced in younger MIS-C patients, but IgG antibodies to HCoV-OC43 and SARS-CoV-2 nucleoproteins were significantly higher than those in age-matched controls (Figures 5C and 5D). As with antibodies to the HCoV-OC43 spike, titers of antibodies to the HCoV-OC43 nucleoprotein were not significantly different between MIS-C patients with prior IVIG treatment and those without ($p = 0.354$, Mann-Whitney rank-sum test).

These experiments revealed that children and adolescents respond differently to coronaviral spikes and nucleoproteins, in terms of magnitude and antibody class. Moreover, across all pediatric and adolescent groups, the antibody responses to the spike and nucleoprotein of each virus were only weakly correlated (correlation coefficients of 0.241 and 0.286 for HCoV-OC43 and SARS-CoV-2, respectively), suggesting differential targeting of either antigen in a given patient or donor. Preferential targeting of SARS-CoV-2 spike rather than nucleoprotein has been linked with a favorable outcome,[29,30] and we therefore examined the ratio of antibodies to spike and nucleoprotein in the patient cohorts. This analysis revealed significantly higher ratios of HCoV-OC43 spike to nucleoprotein antibodies in all disease groups compared with those in the healthy control group, with the exception of older JIA patients (Figure 6A). Higher ratios of spike to nucleoprotein antibodies associated positively with disease, as well as disease activity, and with treatment with steroids, but not with biologics (Figure 6B). Higher ratios of spike to nucleoprotein antibodies for all disease groups and for both HCoV-OC43 and SARS-CoV-2 were confirmed by multiple regression analysis, which also identified significant positive associations with female sex and steroid treatment and a negative association with older age (Figure S2; Table S6). This profile was consistent with lower exposure and a better outcome of infection in these patients.

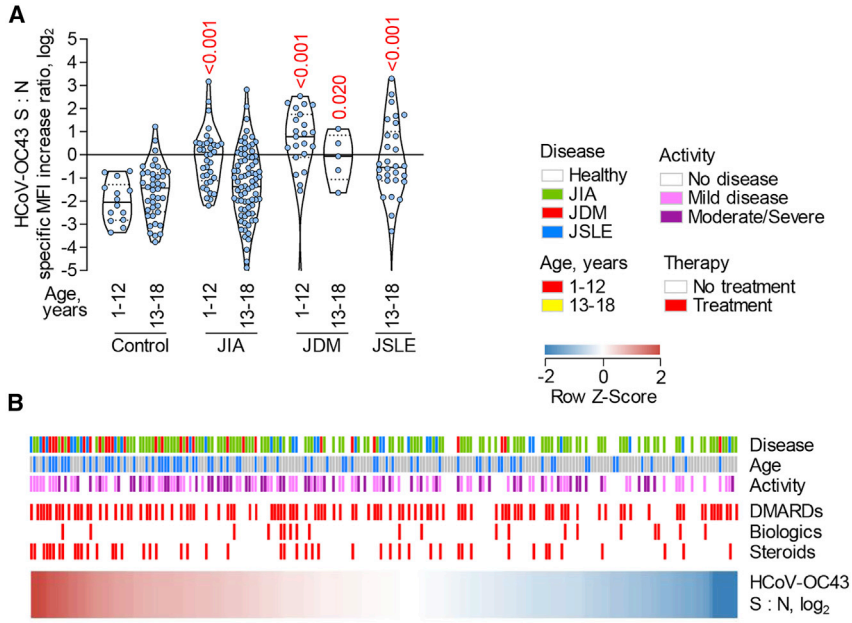

Figure 6. Ratios of the levels of antibodies to coronaviral spikes and nucleoproteins in pediatric and adolescent JIA, JDM, and JSLE patients and age-matched controls

(A) The log2-transformed ratios of total antibodies to the HCoV-OC43 spike to total antibodies to the HCoV-OC43 nucleoprotein (S: N) are plotted for the indicated age and disease group. Each symbol is an individual sample. Numbers within the plots denote the p values of statistically significant increases, when comparing each disease group with the respective healthy control of the same age group.

(B) Heatmap of ranked S: N ratios in the same samples, with each column representing a patient or control. The sample annotations for disease; age; disease activity; and treatment with steroids, biologics, or disease-modifying anti-rheumatic drugs (DMARDs) are also indicated.

DISCUSSION

The current pandemic caused by SARS-CoV-2 infection has highlighted the need for a deeper understanding of the interaction of coronaviruses with the human immune system. Antibody responses, as well as the outcome of SARS-CoV-2 infection, are highly variable among healthy adults, for reasons that remain incompletely understood,[31] and may be even more variable in people with immunological disorders. Here, we show that, despite adaptive immune dysfunction and immunosuppressive treatment, pediatric and adolescent JIA, JDM, and JSLE patients can maintain antibody responses to coronaviruses that are comparable to or stronger than those of healthier peers.

The susceptibility of patients with common rheumatic or other autoimmune diseases to SARS-CoV-2 infection and subsequent disease is still a matter of debate.[32] Studies conducted exclusively in adult cohorts suggested an increased risk of hospitalization due to COVID-19 in autoimmune disease patients, which was attributed to immunosuppressive treatment with glucocorticoids particularly in rheumatic diseases,[16,17,33] an association seemingly at odds with the observed effect of dexamethasone treatment in lowering mortality in patients with the most severe COVID-19 symptoms.[34] Moreover, independent studies indicated that an increased risk of COVID-19 in patients with rheumatic diseases may not be associated with immunosuppressive treatment directly, but rather with disease activity, which requires proportionally higher doses of glucocorticoids.[35] Lastly, even the association between an increased risk of COVID-19 and rheumatic disease may not be direct. A

study of rheumatic disease patients in a region with high COVID-19 prevalence linked the risk of COVID-19 with age and comorbidities, instead of type of rheumatic condition or immunosuppressive treatment.[36]

Rheumatic diseases include conditions with various etiologies and degrees of immune dysfunction, which likely contribute to the complexity of the association with the risk of COVID-19. Nevertheless, the severity of COVID-19 in this group of patients appears much less than was originally expected. Moreover, children are at a much lower risk of COVID-19 than adults in general, and studies of COVID-19 susceptibility in children with rheumatic diseases are currently lacking.

Children and adolescents encounter a number of different respiratory viruses with overlapping epidemiological characteristics. For example, in addition to HCoVs, most children will encounter RSV by the age of 2 and are also likely to become infected with IAV. Despite overlapping age-related prevalence, there appear to be notable differences in the antibody response to such viruses. The response to RSV F decreases with adolescence, likely following reduced infection with age, whereas the response to IAV increases. Moreover, antibody responses to RSV were comparable between JIA, JDM, and JSLE patients and healthier controls, whereas antibody responses to IAV were higher at least in JDM and JSLE patients. These findings contrast with observations of lower rates of seroconversion among patients with SLE than those of healthy controls induced by some, but not all, vaccines.[37,38]

Using the highly prevalent HCoV-OC43, which frequently infects children,[3,11] we found no evidence for a defective antibody response to the spike glycoprotein in JIA, JDM, and JSLE patients. In fact, all other variables considered, such patients often maintained a stronger response to the HCoV-OC43 spike, likely due to immune hyperactivity, despite immunosuppressive treatment.

Similarly to RSV and IAV, exposure to HCoVs starts very early in life and, in most cases, before the onset of autoimmunity in the cohorts we have studied. It is possible that the first HCoV infection set the scene for the maintenance of normal responses to subsequent infections after the onset of disease. Nevertheless, it is also clear that antibody responses to HCoVs are short-lived, providing protection from reinfection for an average of 12 months, and their maintenance depends on repeated infection.[39] Samples were collected from our cohort at a median of 29, 52, and 35 months after the onset of JIA, JDM, and JSLE symptoms, respectively, during which time repeated infections with HCoVs would have boosted antibody titers.[39] Our findings would thus suggest that JIA, JDM, and JSLE patients continued to mount or maintain an appropriate response to HCoVs during disease and its treatment. Our findings are consistent with a study of SARS-CoV-2 infection in a small number of adult rheumatic diseases patients, which detected antibody responses in most of them.[40]

Also consistent with these findings, pre-existing antibodies that cross-react with SARS-CoV-2 spike and cross-neutralize authentic SARS-CoV-2 *in vitro* were also present in pediatric and adolescent JIA, JDM, and JSLE patients, arguing against deficient immunity to coronaviruses. We examined the presence of SARS-CoV-2 cross-reactive antibodies in pre-pandemic samples only as evidence of successful prior responses to HCoVs with broad reactivity. We make no presumption as to the role of HCoV-induced cross-reactive antibodies, memory B cells, or T cells during infection with SARS-CoV-2. Frequently repeated infection with HCoVs in children and adolescents suggests that spike-reactive antibodies do not prevent re-infection

with homologous HCoVs and would, therefore, be highly unlikely to prevent infection with SARS-CoV-2.

Levels of spike-reactive antibodies were also affected, to a certain degree, by the year in which the samples were collected. A recent report provided evidence to suggest an antigenic drift of HCoVs, likely driven by antibody-mediated selection.[22] Indeed, antibodies in historical human serum samples neutralized contemporaneous isolates of HCoV-229E but not future isolates.[22] The relationship between collection year and HCoV-OC43 spike-reactive antibody levels we observed here was not linear, with no overall change over time. This finding does not speak against potential antigenic drift[22] for the following reasons. First, the time interval we analyzed here was relatively shorter, and second, we measured binding antibodies, which recognize a wider range of antigenic epitopes than neutralizing antibodies and, therefore, are less affected by specific mutations.

In contrast to antibodies to coronaviral spikes, those to nucleoproteins were not found elevated in pediatric and adolescent JIA, JDM, and JSLE patients and, in certain groups, were even reduced compared with healthy controls. This finding argues against increased exposure to HCoVs in such patients that could have resulted from relative immune deficiency but rather to a stronger response to the spikes following similar or possible lower exposure.

Interestingly, both pre-existing and *de novo* antibody responses of children and adolescents to SARS-CoV-2 spike are predominantly of the IgG class,[12,28] whereas we found that their responses to the nucleoproteins are dominated by IgM. This feature highlights distinct paths of evolution of the antibody responses to spike and nucleoprotein, with much slower and incomplete class switch for the latter with age. Of note, the dominance of IgM and IgG antibodies in healthy children and the elderly, respectively, were also observed in the response to the nucleoprotein of another seasonal coronavirus, HCoV-229E, in a systems serology study,[41] although this feature extended also to the spike in that study. Potentially related to incomplete class switching of the response to the nucleoproteins is our observation of a much more limited cross-reactivity between HCoV-OC43 and SARS-CoV-2 nucleoproteins than expected from sequence identity. Indeed, antibodies to the nucleoproteins in pre-pandemic sera did not cross-react strongly with the SARS-CoV-2 nucleoprotein, and the back-boosting of antibodies to the HCoV-OC43 nucleoprotein was limited after SARS-CoV-2 infection in MIS-C patients. Although, over their entire length, nucleoproteins and spike proteins display a comparable degree of identity between HCoVs and SARS-CoV-2, the spikes are approximately three times larger than the nucleoproteins and contain distinct regions of high and low conservation, which may explain their increased cross-reactivity.

The early balance of antibodies to SARS-CoV-2 spike and nucleoprotein has been linked with COVID-19 survival, with lower ratios associated with an increased risk of death in adult patients.[29] In an independent study, higher ratios of antibodies to the SARS-CoV-2 spike and nucleoprotein were observed in COVID-19 patients with mild illness than in patients with severe illness.[30] These findings supported a model in which a higher ratio of protective antibodies to spike and non-protective antibodies to nucleoprotein is a hallmark of effective immunity and predicts disease trajectory.[29] In the case of HCoV-OC43, the ratio of antibodies to spike and nucleoprotein was significantly higher in pediatric and adolescent JIA, JDM, and JSLE patients, consistent with protective humoral immunity.

In addition to the antibody response, coronavirus infection induces a T cell response, as well as innate immune responses. T-cell-dependent antibody responses may also reflect an induction of sufficient T cell help during infection but not necessarily other aspects of the T cell response, such as the cytotoxic T cell response. One limitation of the current study is that the T cell responses to HCoV-OC43 or any other HCoVs were not examined. Such responses show higher cross-reactivity among HCoVs and SARS-CoV-2 than antibodies.[42] Abnormal T cell responses are often observed in systemic autoimmune diseases, and their treatment may also adversely affect T cell susbsets.[43,44] It is, therefore, possible that T cell responses to HCoVs are functionally altered in JIA, JDM, and JSLE patients, which could affect T-cell-mediated protection. Similarly, inflammatory rheumatic diseases are often characterized by persistently elevated levels of type I interferons (IFNs), implicated in pathogenesis.[45,46] Recent studies emphasized the important contribution of type I IFNs in preventing severe COVID-19,[47,48] raising the possibility that elevated type I IFN responses in JIA, JDM, and JSLE patients provide a degree of innate immune protection against coronavirus infection.

Although the future of the current pandemic is difficult to project accurately, recent studies suggest SARS-CoV-2 may ultimately become endemic with a low infection fatality ratio, similar to seasonal HCoVs.[49] Interestingly, an analysis of the mutational history of HCoV-OC43 indicated a relatively recent zoonotic introduction into humans,[50] which may have resulted in a major epidemic before HCoV-OC43 became endemic. Therefore, immune responses to current seasonal HCoVs can be informative of the response to SARS-CoV-2, should the latter also become endemic.

Limitations of study

The current study is limited in several aspects. First, due to the paucity of SARS-CoV-2 infection cases in children and adolescents with prevalent inflammatory rheumatic diseases, the response to HCoV-OC43 was examined as a proxy. However, the degree to which responses to HCoV-OC43 accurately predict responses to SARS-CoV-2 remains unclear. HCoVs infect repeatedly, and the antibodies we measured reflect the life history of exposure to HCoV-OC43, without precise knowledge of the timing of the last exposure prior to sampling. In contrast, protection from SARS-CoV-2 will likely depend on a rapid response to acute infection. Moreover, immune responses to SARS-CoV-2 will inevitably be mounted against the backdrop of existing immunity to HCoV-OC43 and other HCoVs. Although the cross-reactivity of antibodies and T cells with HCoVs and SARS-CoV-2 is demonstrated, its effect is still incompletely understood. Several studies have now suggested protective,[51–55] damaging,[56–59] or neutral[20,60] effects of HCoV-induced pre-existing immunity on SARS-CoV-2 infection and disease, but a conclusive answer will require further investigation.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Human subjects

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.medj.2021.08.001.

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AUTHOR CONTRIBUTIONS

S.J.G., J.M., E.N., M.L., P.C., E.C.R., C.C., L.R.W., and G.K. conceived, designed, and supervised the study. B.R.J., M.G.L.W., L.R.M., K.O., A.R., H. Peckham, H. Patel, J. Heaney, H.R., S.P., C.F.H., and M.J.S. collected patient and donor samples. P.C., C.E., A.R., and J. Hope provided reagents. G.H.C., K.W.N., N.F., W.B., R.H., and S.H. performed assays and acquired data. C.T.D. and G.K. had unrestricted access to all data, analyzed and interpreted data, and performed statistical analyses. C.T.D., C.C., L.R.W., and G.K. prepared the first draft of the manuscript, reviewed it, and edited it. All authors agreed to submit the manuscript, read and approved the final draft, and take full responsibility of its content.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
CR3022	Absolute Antibodies	Cat# Ab01680-10.0; RRID: AB_2848080
D001	SinoBiological	Cat# 40590-D001; RRID: AB_2857932
BV421 anti-IgG	Biolegend	Cat# 409318; RRID: AB_2562176
APC anti-IgM	Biolegend	Cat# 314510; RRID: AB_493011
PE anti-IgA	Miltenyi Biotech	Cat# 130-114-002; RRID: AB_2733860
PE anti-IgG1	Southern Biotech	Cat# 9054-09; RRID: AB_2796628
HRP-conjugated goat anti-human IgG	Invitrogen	Cat# A18805; RRID: AB_2535582
Virus strains		
SARS-CoV-2 isolate hCoV-19/England/02/2020	Respiratory Virus Unit, Public Health England, UK	GISAID EpiCov™ accession EPI_ISL_407073
Biological samples		
Human serum or plasma	This study	N/A
Chemicals, peptides, and recombinant proteins		
SARS-CoV-2 nucleoprotein	Ng et al.[12]	N/A
HCoV-OC43 nucleoprotein	This study	N/A
SARS-CoV-2 S2 subunit	LifeSensors	Cat# CV2006
RSV F protein	SinoBiological	Cat# 40627-V08B
IAV H1N1 HA protein	SinoBiological	Cat# 11085-V08H
Experimental models: Cell lines		
HEK293T	Cell Services facility at The Francis Crick Institute	CVCL_0063
Vero E6	Cell Services facility at The Francis Crick Institute	CRL-1586
SUP-T1	Cell Services facility at The Francis Crick Institute	CRL-1942
Recombinant DNA		
pRV-HERV-K113_env-IRES-GFP	Ng et al.[12]	N/A
pRV-ERV3-1_env-IRES-GFP	This study	N/A
pRV-SARS-CoV-2_spike-IRES-GFP	This study	N/A
pcDNA3- SARS-CoV-2_spike	Gift from Dr. Massimo Pizzato, University of Trento, Italy	N/A
pcCMV3- HCoV-OC43_spike	SinoBiological	Cat# VG40607-CF
Software and algorithms		
Bio-Rad Everest	Bio-Rad	v2.4
FlowJo	Tree Star Inc.	v10
SigmaPlot	Systat Software	v14.0
GraphPad Prism	GraphPad	v8
LabVIEW	National Instruments	N/A
R	https://www.r-project.org	v4.0.2

RESOURCE AVAILABILITY

Lead contact

All unique/stable reagents generated in this study are available from the Lead Contact with a completed Materials Transfer Agreement.

Materials availability

Plasmid and unique cell lines generated here are available upon request, as collaboration.

Data and code availability

- Fully anonymized data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.

- Any additional information required to reanalyze the data reported in this paper is available from the Lead Contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Human subjects

Pre-pandemic serum or plasma samples from children and adolescents were obtained from the Centre for Adolescent Rheumatology Versus Arthritis at University College London (UCL), University College London Hospitals (UCLH), and Great Ormond Street Hospitals (GOSH), and UCL Great Ormond Street Institute for Child Health (ICH) with ethical approval (study references 11/LO/0330, 99RU11, 04RU07 and 95RU04) (Table S1). For statistical analyses, gender was considered as assigned at birth, to reflect chromosomal influences on adaptive immunity. Healthy children and adolescents aged 6-16 years were recruited at pre-assessment appointments for elective dental or urological surgery, and healthy teenagers aged 16-18 years were recruited at "Young Scientist Days" at the Centre for Adolescent Rheumatology. Individuals were screened out if they or their parents reported having a condition that may affect their immune system e.g., serious infections or recent vaccination. Some healthy donors had a confirmed or suspected family history of autoimmune disease (n = 3) or a sibling with JIA (n = 3). Pre-pandemic samples from adults were obtained from UCLH (study ref 284088). These samples were from residual samples prior to discarding, in accordance with Royal College Pathologists guidelines and the UCLH Clinical Governance for assay development and GOSH and ICH regulations. Patients with MIS-C were recruited through the DIAMONDS (Diagnosis and Management of Febrile Illness using RNA Personalised Molecular Signature Diagnosis) study at Imperial College Healthcare NHS Trust. Patients meeting the WHO criteria for MIS-C[15] were recruited at St Mary's Hospital, Imperial College Healthcare NHS Trust, London, with ethical approval (Research Ethics Committee reference 20/HRA/1714). MIS-C patients had a median age of 10.4 (range 1.8-14.7) and seropositivity for SARS-CoV-2 spike antibodies. Of the 14 recruited MIS-C patients, 5 had received intravenous immunoglobulin (IVIG) prior to sampling, 8 had not received IVIG prior to sampling, and this information was not available for 1 patient. All samples were obtained with formal consent from patients, donors or their families. Samples had undergone at least one cycle of thaw and freeze, and had been stored at either −20°C or −80°C freezers at local hospitals prior to transfer to the Francis Crick Institute. All serum or plasma samples were heat-treated at 56°C for 30 min prior to testing.

Demographic and clinical data were obtained via the Centre for Adolescent Rheumatology Versus Arthritis, the Childhood Arthritis Response to Medication Study (CHARMS), the JIA Pathogenesis Study, the JDM Cohort & Biomarker Study databases, and medical records. For the healthy children and adolescents, demographic details and medication data were recorded, however, clinical laboratory tests performed during routine assessment for rheumatic diseases were not performed in these individuals. Since certain items of clinical data were recorded differentially across disease cohorts or were not recorded for healthy children and adolescents, the following assumptions were applied in order to maximize complete data for the regression analyses. Patients were treated as being 'autoantibody positive' if any of rheumatoid factor (RF), anti-nuclear antibodies (ANA) or myositis-specific/myositis-associated autoantibodies were recorded as positive. Healthy controls were not tested for RF/ANA or myositis autoantibodies and although a low rate of low titer ANA can be detected in healthy individuals, these individuals were assumed autoantibody-negative for the purpose of this analysis. Healthy children and adolescents were not on steroids, biologics, disease modifying anti-rheumatic drugs

(DMARDs) or other immunosuppressants at time of sampling. Disease activity in patients had been recorded using either the SLE disease activity index (SLEDAI) for JSLE or a physician's visual analog scale (PhysVAS) for JIA and JDM. A categorical disease activity variable was defined as "no disease" (SLEDAI = 0 or PhysVAS = 0), "mild" (0 < SLEDAI $\leq$ 4 or 0 < PhysVAS $\leq$ 3.5) or "moderate to severe" (SLEDAI > 4 or PhysVAS > 3.5). Healthy controls were included in the "no disease" group. Disease duration was discretised as "no disease/diagnosis" for samples from healthy controls or from patients at diagnosis, "$\leq$24 months" or ">24 months," with the 24 month cut-off selected according to the distribution of disease duration.

METHOD DETAILS

Cell lines and plasmids

HEK293T cells and Vero E6 were obtained from the Cell Services facility at The Francis Crick Institute and verified as mycoplasma-free. HEK293T cells were validated by DNA fingerprinting. Cells were grown in Iscove's Modified Dulbecco's Medium (Sigma Aldrich) supplemented with 5% fetal bovine serum (Thermo Fisher Scientific), L-glutamine (2 mM, Thermo Fisher Scientific), penicillin (100 U/ml, Thermo Fisher Scientific), and streptomycin (0.1 mg/ml, Thermo Fisher Scientific).

HEK293T cells expressing HERV-K113 envelope glycoprotein were generated by retroviral transduction with the vector encoding the putative ancestral protein sequence of HERV-K113 envelope glycoprotein[61] and GFP separated by an internal ribosome entry site (IRES). HEK293T cells expressing ERV3-1 envelope glycoprotein were similarly generated by retroviral transduction with the vector encoding the ERV3-1 envelope glycoprotein (NCBI Reference Sequence: NM_001007253.4) and GFP separated by an IRES. Transduced cells were sorted based on GFP expression to > 98% purity on a FACSAria Fusion cell sorter (Beckton Dickinson) and maintained as separate cell lines.

HEK293T cells expressing SARS-CoV-2 or HCoV-OC43 spikes were generated by transient transfection, using GeneJuice (EMD Millipore), with expression plasmids, encoding each spike and were used two days after transfection. The expression vector (pcDNA3) carrying a codon-optimized gene encoding the wild-type SARS-CoV-2 spike (UniProt ID: P0DTC2) was kindly provided by Massimo Pizzato, University of Trento, Italy. The expression vector (pCMV3) carrying a codon-optimized gene (NCBI Reference Sequence: AVR40344.1) encoding the HCoV-OC43 spike of isolate HCoV-OC43/USA/TCNP_00212/2017 was obtained from SinoBiological. SUP-T1 (CRL-1942) T cell lymphoblastic lymphoma cells were transduced with a pRV-based retroviral vector carrying a codon-optimized gene encoding the wild-type SARS-CoV-2 spike (UniProt ID: P0DTC2) and a gene encoding GFP, separated by an IRES. Expression of SARS-CoV-2 spike was detected by staining with the CR3022 antibody (Absolute Antibodies) or D001 antibody (40590-D001, SinoBiological). GFP^+ SARS-CoV-2 $Spike^+$ cells were purified by cell sorting to establish a stable cell line, SUP-T1.spike.

Flow cytometric detection of antibodies to spike and envelope glycoproteins

HEK293T cells expressing HCoV-OC43 spike were mixed with cells expressing ERV3-1 envelope glycoprotein and GFP and HEK293T cells expressing SARS-CoV-2 spike were mixed with cells expressing HERV-K113 envelope glycoprotein and GFP at equal rations (Figure S1). All cells were tryspinised prior to mixing and cell mixtures were dispensed into V-bottom 96-well plates (20,000-40,000 cells/well). Cells were incubated with sera (diluted 1:50 in PBS) for 30 min, washed with FACS buffer (PBS, 5% BSA, 0.05% sodium azide), and stained with BV421 anti-IgG

(clone HP6017, Biolegend), APC anti-IgM (clone MHM-88, Biolegend) and PE anti-IgA (clone IS11-8E10, Miltenyi Biotech) for 30 min (all antibodies diluted 1:200 in FACS buffer). Cells were washed with FACS buffer and fixed for 20 min in CellFIX buffer (BD Bioscience). Samples were run on a Ze5 analyzer (Bio-Rad) running Bio-Rad Everest software v2.4 and analyzed using FlowJo v10 (Tree Star Inc.) analysis software, as previously described.[12] Specific increase in staining intensity by sample antibodies was calculated by comparing the mean fluorescence intensity (MFI) of each of the four HEK293T cell types stained with serum or plasma samples for each Ig class with the MFI of the respective unstained HEK293T cell type, using the following formula: (MFI of stained cells − MFI of unstained cells)/MFI of unstained cells. Samples with values over 0.5 were considered positive for binding antibodies.

Affinity of serum antibodies for SARS-CoV-2 spike was assessed based on a previously described flow cytometric method.[27] To this end, SARS-CoV-2 spike-expressing SUP-T1.spike cells were mixed in equal ratios with parental SUP-T1 cells, which served as an internal negative control (Figure S5). Cell mixtures were dispensed into V-bottom 96-well plates (20,000-40,000 cells/well). Cells were incubated with sera (diluted 1:50 in PBS) for 60 min, washed three times with FACS buffer (PBS, 5% BSA, 0.05% sodium azide) and kept on ice. Replicate wells were then incubated at 37°C for 1, 2, 5 or 10 minutes and returned to a cold temperature. All cells were subsequently stained with PE anti-IgG1 (1:500; clone HP6001; Southern Biotech #9054-09) and fixed before they were analyzed by flow cytometry. Specific MFI increases were calculated using the same formula as for HEK293T cells. Specific staining over the incubation time was expressed as a percentage of the maximum staining and was considered proportional to the antibody off-rate.[27]

Recombinant protein production

Full-length SARS-CoV-2 and HCoV-OC43 nucleoproteins were produced by expression in *Escherichia coli* C43(DE3) cells (Lucigen) and purified as previously described.[12] The SARS-CoV-2 nucleoprotein was produced with an N-terminal His_6 tag from pOPTH-1124 plasmid (kindly provided by Jakub Luptak and Leo James, Laboratory for Molecular Biology, Cambridge, UK). The HCoV-OC43 nucleoprotein (NCBI Reference Sequence: AOL02457.1 from isolate HCoV-OC43/human/Mex/LRTI_238/2011) was produced with an N-terminal SUMO-His_6 tag, which was removed during purification, by incubation with ubiquitin-like-specific protease-1 (ULP1) at 4°C overnight. Following incubation, the protein was diluted to 300 mM NaCl in 20 mM HEPES pH 8.0, loaded onto a 5 mL HiTrap heparin column (GE healthcare) and eluted over a linear 0.3-1M NaCl gradient. Purified nucleoproteins were concentrated by ultrafiltration using appropriate VivaSpin devices (Sartorius), were snap-frozen in liquid nitrogen in small aliquots and stored at −80°C.

Flow cytometric detection of antibodies to nucleoproteins

Aliquots of recombinant HCoV-OC43 and SARS-CoV-2 nucleoproteins were conjugated with aldehyde functionalized polymethylmethacrylate-based microspheres (PolyAn GmbH, Berlin, Germany), according to manufacturer's instructions, and kept at 4°C until use. Transparent beads of 5 μM and 2 μM diameter were conjugated with HCoV-OC43 and SARS-CoV-2 nucleoproteins, respectively (Figure S1). Beads were mixed at equal ratio and were dispensed into V-bottom 96-well plates (a total of 50,000-70,000 beads/well). Beads were then stained with serum and plasma samples, as described above for HEK293T cells used in flow cytometric detection of antibodies to spike and envelope glycoproteins. Specific increase in staining intensity by sample antibodies was calculated by comparing the mean

fluorescence intensity (MFI) of each type of bead stained with serum or plasma samples for each Ig class with the MFI of the respective unstained bead type, using the following formula: (MFI of stained beads − MFI of unstained beads)/MFI of unstained beads. Samples with values over 0.5 were considered positive for binding antibodies.

ELISA for detection of antibodies to respiratory viruses

ELISAs were run as previously described[12] with the exception that the following proteins were used from coating: SARS-CoV-2 S2 subunit ($S_{686-1211}$, CV2006, LifeSensors), RSV F protein (40627-V08B, SinoBiological), and IAV H1N1 HA protein (11085-V08H, SinoBiological). Following incubation with serum dilutions (1:50), plates were washed and incubated with horseradish peroxidase-conjugated goat anti-human IgG (1:2000; Invitrogen #A18805) for 1 hour. Plates were developed by adding 50 µl of TMB substrate (Thermo Fisher #N301), followed by 50 µl of TMB stop solution (Thermo Fisher #N600) after 5 mins shaking at room temperature, and optical densities (ODs) were measured at 450 nm on a microplate reader (Tecan). Results were expressed as fold-change of OD readings between samples and no serum control wells.

SARS-CoV-2 neutralisation assay

Titers of SARS-CoV-2 neutralising antibodies were determined as previously described.[12] Briefly, the SARS-CoV-2 isolate hCoV-19/England/02/2020 was obtained from the Respiratory Virus Unit, Public Health England, UK (GISAID EpiCov™ accession EPI_ISL_407073) and propagated in Vero E6 cells. Triplicate cultures of Vero E6 cells were incubated with SARS-CoV-2 and twofold serial dilutions of human sera (previously heat-treated at 56°C for 30 min) for 3 hours. The inoculum was then removed and cells were overlaid with virus growth medium containing 1.2% Avicel (FMC BioPolymer). At 24 hours post-infection, cells were fixed in 4% paraformaldehyde and permeabilized with 0.2% Triton X-100/PBS, and virus plaques were visualized by immunostaining.[12] Virus plaques were quantified and IC_{50} values were calculated using LabVIEW software as described previously[62] or SigmaPlot v14.0 (Systat Software).

QUANTIFICATION AND STATISTICAL ANALYSIS

Data were analyzed and plotted in GraphPad Prism v8 (GraphPad Software) or SigmaPlot v14.0 (Systat Software). Parametric comparisons of normally-distributed values that satisfied the variance criteria were made by unpaired Student's t tests or One Way Analysis of variance (ANOVA) tests. Data that did not pass the variance test were compared with non-parametric two-tailed Mann-Whitney Rank Sum tests or ANOVA on Ranks tests. Prevalence of seropositivity was compared with Fisher's exact tests, with Bonferroni correction for multiple comparisons.

Regression analyses were performed in order to quantify the effects of disease group and adjust for the effects of demographic and other clinical variables. Univariate analyses of each clinical and demographic variable were performed initially to identify which predictor variables displayed an association with each of the outcome variables. These analyses described the size of the effect of each predictor variable on antibody levels and whether that effect was statistically significant. Multiple regression analyses were then performed to identify the model that explained the greatest amount of variability in antibody levels using disease group, age, gender and any other clinical or demographic variables that had statistically meaningful effects. Multiple regression analyses started with a base model comprising disease group, age and gender to ensure age and gender effects were always controlled

for. The other demographic and clinical predictor variables were added in sequentially, with likelihood ratio tests (LRTs) used to determine which additional variables significantly added to the model. This process ensured that any confounding variables were appropriately controlled for. Regression analyses were performed using R v4.0.2[63] and the "lm" function in the base statistics package. LRTs were performed using the "lrttest" function in the lmtest package v0.9-38.[64] Residual plots were checked to confirm the regression assumptions of linearity and homoscedasticity were met.

Predictor variables considered in regression models were: disease group, age, gender, year of sample, disease duration, disease activity, steroids, biologics, DMARDs and other immunosuppressants, and autoantibody. Age was discretised as < 13 or ≥ 13 based on the distributions of the antibody data by age and the lack of samples from JSLE patients under 13 years of age. All predictor variables were categorical, as described above. Outcome variables modeled were the total, IgG and IgA antibody levels for SARS-CoV-2 and OC43 spike proteins and nucleoproteins. A square-root transformation was applied to the outcome variables in order to better meet assumptions of linearity in regression. Estimates and 95% confidence intervals (CIs) are reported on the square-root scale and do not relate antibody levels on the original scale, however, the antibody levels represent relative fluorescence and do not have meaningful units anyway. The sign of the estimates and the magnitude of estimates within categorical variables are meaningful.