Humoral cross-coronavirus responses against the S2 region in children with Kawasaki disease

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ABSTRACT

Multisystem Inflammatory Syndrome in Children (MIS-C), a post infectious complication of SARS CoV-2 infection, shares enough features with Kawasaki Disease (KD) that some have hypothesized cross-coronavirus (CoV) immunity may explain the shared pathology. Recent studies have shown that humoral cross-reactivity of the CoVs, particularly of OC43, is focused on the S2 region of the Spike protein.

Due to efforts utilizing CoV S2 regions to produce a cross-CoV vaccine, we wished to assess SARS-CoV-2 S2 reactivity in children with KD and assess if cardiac involvement in KD correlated with S2 CoV antibody targeting. The presence of cross-reactivity does not distinguish KD from febrile controls and does not correlate with cardiac involvement in KD. These findings support that, in relation to cardiac vascular inflammation, vaccines targeting the S2 region appear to be a safe approach, but there is disparity in the ability of CoV species to raise cross-reactive S2 targeted antibodies.

1. Introduction

Cross-coronavirus (CoV) vaccines are being pursued, but lack of knowledge of immune related conditions related to SARS-CoV-2, such as Multisystem Inflammatory Syndrome in Children (MIS-C), necessitates caution. Class I fusion proteins, utilized in viral families Arenaviridae, Coronaviridae, Filoviridae, Orthomyxoviridae, Paramyxoviridae, and Retroviridae, form trimers that contain a region that facilitates recognition of the targeted host cellular receptor and a region that enables cell and viral membrane fusion. (Schibli and Weissenhorn, 2004). For Coronaviridae, the S2 region enables fusion and contains two heptad repeat (HR) regions that have significant alpha-helical structure (Monteiro et al., 2021). The S2 folds to approximate the alpha-helical HR regions to form a trimer of helical hairpins termed a six-helix bundle (6HB) (White et al., 2008), which is crucial for approximating the two membranes leading to fusion (Schibli and Weissenhorn, 2004; White et al., 2008; Eckert and Kim, 2001; Skehel and Wiley, 2000; Ding et al., 2017).

The common cold human CoVs (hCoVs) can be divided into alpha (CoV-229E and CoV-NL63) and beta (CoV-HKU1 and CoV-OC43). Cross-CoV humoral immunity has been demonstrated (Ng et al., 2020) and is likely explained by prior immunity to circulating hCoVs (Grifoni et al., 2020; Shrock et al., 2020). Pre-pandemic samples with detectable levels of SARS-CoV-2 reacting antibodies had higher levels of antibodies against the hCoV-OC43 S protein and specifically the S2 segment (Anderson et al., 2021a). Prior immunity to circulating hCoVs is boosted after SARS-CoV-2 infection (Anderson et al., 2021a). A number of studies suggest prior immunity to circulating hCoVs may contribute to SARS-CoV-2 neutralization (Dugas et al., 2021a; Galipeau et al., 2021). The cross reactivity between SARS-CoV-2 and other circulating hCoVs is predominantly based on S2 region cross-reactivity (Wolfel et al., 2020; Nguyen-Contant et al., 2020), including Cross-CoV neutralizing antibody responses (Jennewein et al., 2021). Studies have shown that the structure of the C-terminal HR region of the S2 region is particularly conserved among the CoV species (Xia et al., 2020). On comparative sequence analysis, hCoV-OC43 has significant homology in the S2 region in comparison to other hCoVs (Monteiro et al., 2021; Nguyen-Contant et al., 2020; Anderson et al., 2020; Beretta et al., 2020). A peptide fusion inhibitor developed from the OC43 hCoV CHR has even shown inhibition against other CoV species (Xia et al., 2019), including SARS-CoV-2 (Xia et al., 2020). Humoral immunity to OC43 in particular has been associated with potential cross-neutralization and improved clinical outcomes (Dugas et al., 2021a, 2021b).

Early reports noted the similarity of SARS-CoV-2 related MIS-C to Kawasaki Disease (KD) (Riphagen et al., 2020; Verdoni et al., 2020; Hennon et al., 2021). KD is a vasculitis and major cause of childhood acquired heart disease in the pediatric population. Epidemiological

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Abbreviations[1]		hCoV	human coronavirus
		hRBD	human expressed proteins of Receptor Binding Domain
OC43-bS	baculovirus expressed constructs of the OC43 Spike protein	hTrimS	human expressed trimerized Spike protein
		KD	Kawasaki Disease
OC43-bS2	baculovirus expressed constructs of the OC43 Spike S2 region	KDB	Kawasaki Disease Biorepository
		LAD	left anterior descending
bS	baculovirus expressed constructs of the SARS-CoV-2 Spike protein	LCA	left circumflex artery
		LMCA	left main coronary artery
bS2	baculovirus expressed constructs of the SARS-CoV-2 Spike S2 region	MISC	Multisystem Inflammatory Syndrome in Children
		NP	nucleocapsid protein
chRBD	commercially available human expressed Receptor Binding Domain	PBMC	peripheral blood mononuclear cell
		RCA	right circumflex artery
CoV	Coronavirus	6HB	six-helix bundle
FC	febrile controls	S	Spike protein
HR	heptad repeat- HR	UBKD	University at Buffalo KD subject samples

patterns suggest KD is related to an infectious agent; however, the etiology remains unknown (McCrindle et al., 2017). Prior reports have suggested CoVs can cause KD (Shirato et al., 2014; Esper et al., 2005). We have previously addressed whether targeting of the RBD epitope, full Spike protein, or nucleoprotein could explain the similar pathology seen in children with KD and post-acute SARS-CoV-2 MIS-C. A number of prepandemic samples from febrile children had significant increase in binding to SARS-CoV2 nucleocapsid protein (NP) and Spike (S) antigens; however, cross-reactivity did not appear to be elevated in children with Kawasaki disease (Chang et al., 2020).

Recent studies suggest vaccination will prevent cases of MIS-C (Levy et al., 2022; Zambrano et al., 2022). Although MIS-C does not appear to be associated with immune reactivity to the Spike protein (Chang et al., 2020), the cases of post-vaccination myocarditis with the mRNA vaccines remain unexplained (Oster et al., 2022). Higher rates associated with the mRNA vaccines suggest that the platform plays a significant role in induction of these cases of post-vaccination myocarditis. As broad spectrum hCoV fusion inhibitors (Wang et al., 2021) and vaccine approaches (Jennewein et al., 2021) are targeting the S2 region and COVID-19-associated illnesses and vaccinations may be associated with myocarditis, we sought to further explore cross-CoV immunity in groups of children with KD and other febrile pediatric patients with a focus on the S2 region and association with cardiac complications.

2. Methods

2.1. Proteins utilized

Human expressed proteins of Receptor Binding Domain (hRBD; Synthetic construct SARS_CoV_2RBD_his gene; GenBank: MT380724.1) and Trimerized S (hTrimS; Synthetic construct SARS_CoV_2_ectoCSPP gene; GenBank: MT380725.1) were expressed utilizing expression vectors from Florian Krammer's laboratory in 293 F cells following published protocol (Amanat et al., 2020). The sequence is based on the first published isolate Wuhan-Hu-1 (Wu et al., 2020). The following commercial reagents expressed in Baculovirus were obtained from Sino Biological: Nucleocapsid Protein (NP) (Cat # 40588-V08B), S Protein, S1+S2 ECD (Cat#40589-V08B1), SARS-CoV-2 S2 subunit (Cat # 40590-V08B), hCoV-OC43 whole S protein (Cat# 40607-V08B) and hCoV-OC43 S2 subunit (Cat# 40607-V08B1). The Sino Biological human expressed protein RBD (Cat#40592-V08H) was also used for comparison.

2.2. Patient cohorts

Plasma samples from inpatients with fever and respiratory distress who were tested and were SARSCoV-2 polymerase chain reaction (PCR) positive were used as the positive control for normalization of ELISA results. These were collected with IRB approval (University at Buffalo (UB) STUDY00004340) utilizing State University of New York Research Seed Grant Program as previously described (Chang et al., 2020). Plasma samples from 123 febrile controls (FC) and 26 febrile children diagnosed with KD (UBKD), 24 of which had pre-IVIG samples, and their associated clinical information were collected under approval of the UB IRB STUDIES- 00000126, 00002824 and 00005262 with funding support by the Wildermuth MemorialFoundation as previously described (Martin et al., 2018). During peripheral blood mononuclear cell (PBMC) isolation for a separate study, plasma was withdrawn and saved codified in a −80 °C freezer and associated deidentified clinical information was retained. Additional serum samples (30 subjects with pre-treatment, post-treatment, and convalescent samples) were obtained through the Pediatric Heart Network and stored in the Kawasaki Disease Biorepository (KDB) at Boston Children's Hospital (IRB X10-01-0308) collected for a prior study (Newburger et al., 2007). The subset of KDB subjects used herein met complete KD criteria and were treated with intravenous immunoglobulin (IVIG). For UBKD sample, the KD diagnosis was assigned if infectious disease consultation agreed with KD as likely diagnosis, KD was agreed by separate infectious disease specialist on post-hoc review, and patient received IVIG. Other clinical group final diagnoses were defined by post-hoc review of EMR documents including all available laboratories and follow-up assessments. Statistical analysis was performed using GraphPad Prism 9 and groups were compared with Wilcoxon ranked sum tests and Pearson R correlations.

Z scores were calculated using the downloadable Kobayashi Coronary Z-score Calculator available from http://raise.umin.jp/zsp/calculator/(Kobayashi et al., 2016) and the Boston Z-score calculator at https://zscore.chboston.org for both the UBKD and KDB cohorts for comparison. Briefly, patient height, weight, gender, and measured coronary arterial diameter for RCA, LMCA, LAD and LCx for the Kobayashi score were transcribed into appropriate columns in the calculator. Body surface area and Z scores for each coronary artery were then automatically calculated.

3. ELISA

The ELISA protocol used followed a recently published SARS-CoV-2 related protocol (Stadlbauer et al., 2020) with minor adjustments (use of Goat Anti-Human Ig-HRP (Southern Biotech) secondary and TMB Ultra (Thermo Fisher) as developer. Generally, 96-well ELISA plates were coated with proteins at 10 ng/well. Plates were then sealed and incubated overnight at 4 °C. Plates were washed three times with wash buffer (1x PBS, 0.05%Tween-20). All wells were then blocked with 100ul of 5% milk in PBS and incubated at room temperature for 1 h.

Plates were then aspirated and washed once with wash buffer. 50ul of diluted plasma samples of 1:50 in 1% milk PBST were added to each well and incubated at room temperature for 1 h. Plates were then aspirated and washed three times. Goat Anti-Human Ig-HRP (Southern Biotech), diluted in1% milk in PBST, was added and incubated at room temperature for 1 h. Plates were then aspirated, washed three times, and developed with TMB solution with acid stop prior to optical density reading at 450 nm with background plate reading at 630 nm subtracted. For disparate results, select antigen ELISAs were run in triplicate with outliers greater than 2-fold difference from average of other sample results removed.

4. Results

4.1. Cross-reactivity to SARS-CoV-2 in prepandemic febrile children

Samples collected at UB from 2013 to 2018 were used to assess cross-reactivity of circulating immunoglobulin. Of the 26 children with KD enrolled (designated UBKD), the majority (18) fulfilled the diagnostic criteria for complete KD and only two had significantly high Z scores of coronary arteries on echocardiogram (Table 1). Antigen binding was normalized to SARS-CoV-2 infected control samples on each plate to facilitate intra-antigen and inter-antigen comparisons across plates. In our initial study, cross-reactivity for NP was particularly evident in a number of febrile controls (FC) (Chang et al., 2020). In expanding our sample numbers from 36 to 123 FC, ranked sum comparison did not show significant differences (p = 0.4824) (Fig. 1A). There were a number of cross-reactive FC samples on NP, with 21 of 123 having over 10% above background and 3 of 123 over 20% of background in comparison to only one of our KD was above 10%. Likely this reflects a number of infections with pre-pandemic circulating CoVs within our FC cohort.

To explore differences in antigen recognition, we expressed trimerized S protein (hTrimS) in human cells (Amanat et al., 2020) to compare to commercially available baculovirus expressed S constructs (bS). In comparing the hTrimS to the bS constructs using pre-IVIG samples, the bS constructs showed higher cross reactivity for both clinical groups (KD p = 0.0007; FC p = 0.0011) on ranked-sum comparison. A subset of higher bS binding samples were used in the hTrimS assays and are shown in direct comparison (Fig. 1B); only 1 of 40 showed higher binding to hTrimS in normalized binding comparison. This is consistent with previously published data that showed high specificity of hTrimS binding using adult samples, and higher specificity of human cell expressed proteins versus baculoviral proteins (Amanat et al., 2020). As the trimerized protein showed less cross reactivity, this implies that monomeric construct display epitopes that may be shielded when in trimeric forms.

Similar binding was shown with KD samples (chRBD vs. hRBD; p = 0.7866) on commercially available human expressed RBD (chRBD) and human expressed RBD (hRBD) produced in our laboratory. Comparison between those post-IVIG samples also showed no differences (chRBD vs. hRBD; p = 0.9334). In comparison of the FC group, there did appear to be higher cross-reactivity to the hRBD (chRBD vs. hRBD; p < 0.0001), suggesting if there was bias introduced by our methods, it is in the

Table 1
Summary of clinical Aincal characteristics and Coronary Artery Z score

University at Buffalo (UBKD)				Kawasaki Disease Biorepository (KDB)						
Plasma UBKD#	Age (years)	Complete KD?	Z > 2.5	Serum KDB#	Age (years)	Complete KD?		Kobayashi Z = 2-2.5 (19)	Boston Z > 2.5 (8)	Boston Z = 2-2.5 (11)
01	5.8	yes		10021	4.9	yes	LAD		LAD	
02	2.7	no		10031	2.9	yes				
03	2.3	no		10045	3.2	yes				
04	6.5	yes		10054	6.7	yes				
05	4.1	no		10060	2.5	yes	LMCA			
06	4.8	yes		10103	3.2	yes		LMCA, RCA		
07	6.8	yes		10112	4.6	yes		LMCA		
08	2.4	yes		10140	0.3	yes	LAD, LMCA, RCA	LCA	LAD, LMCA	RCA
09	0.8	yes		10184	5.5	yes				
10	1.8	yes		10196	7.8	yes	LMCA			
11	1.7	yes		10262	6.8	yes				
12	2.2	yes		10270	4.9	yes				
13	4.4	yes		10298	4	yes		LMCA		
14	6.9	yes		10351	0.2	yes	LAD, LMCA, RCA		LAD, LMCA, RCA	
15	4.5	no		10369	2.8	yes	LCA, LMCA, RCA	LAD	RCA	LAD
16	3.3	no		10376	6	yes				LAD
17	1.9	no		10396	1.5	yes	RCA			RCA
18	2.4	yes		10397	1	yes				
19	1.0	no		10400	1.1	yes	LCA, LMCA	LAD	LAD	LMCA
20	3.3	yes	RCA	10442	0.2	yes	LAD, LCA, LMCA, RCA		LAD, LMCA	RCA
21	6.9	yes		10450	3.8	yes		RCA		LAD
22	1.6	yes		10477	4.1	yes		LMCA		
23	2.5	yes		10515	4.5	yes				
24	1.0	yes	LCA, RCA	10553	4	yes				
25	4.5	yes		10557	5.5	yes	LAD, LMCA, RCA		LAD, LMCA, RCA	
26	1.3	no		10560	4.8	yes	LMCA			
				10561	1.6	yes		LMCA		
				10571	4.2	yes		RCA		
				10573	0.9	yes	LMCA	LAD	LAD, LMCA	
				10588	5.5	yes				

Kawasaki Disease- KD; left anterior descending- LAD; left circumflex artery- LCA, left main coronary artery- LMCA; right circumflex artery- RCA.
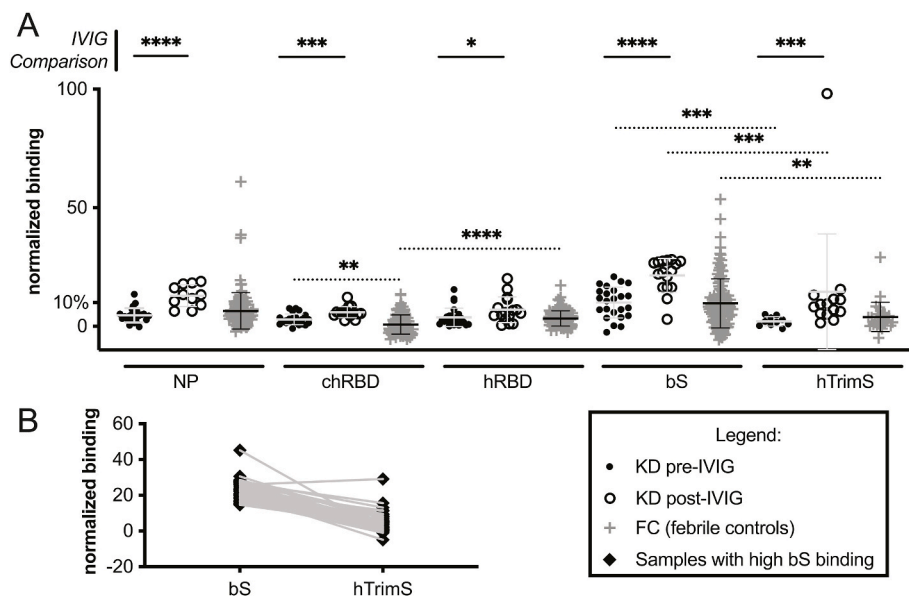
Fig. 1. SARS-CoV-2 protein binding of FC compared to children with KD. Plasma samples from 123 FC were compared to samples from 24 children with KD. To facilitate intra-antigen comparison, optical density at 450 nm results were normalized by subtracting plate background and comparing positive control set at 100% and means of two experiments were compared. A) Solid significance lines are pre and post IVIG comparisons for each antigen. Dotted lines show other significant comparisons (noted in text). B) Diamonds represent samples from all three cohorts that showed higher bS binding that were used in hTrimS assays, with connecting grey lines showing direct comparative samples. Statistical comparison was done with Wilcoxon ranked sum tests with p value summaries: * < 0.05 to 0.01, ** 0.009 to 0.001, *** 0.0009 to 0.0001and **** <0.0001. Error bars represent means with standard deviations.

opposite direction to the results seen for S comparison. Administration of IVIG to patients led to increased reactivity in ELISAs. All comparisons of sample cohorts pre-IVIG and post-IVIG were significant to varying degrees (NP p < 0.0001; chRBD p = 0.003; hRBD p = 0.0160; bS p < 0.0001; hTrimS p = 0.0002).

4.2. SARS CoV S2 region targeting

We explored cross-reactivity of SARS-CoV-2 concentrating on the S2 region and utilizing OC43 to confirm this cross-reactivity in the prepandemic plasma samples. We obtained baculovirus expressed constructs of the S of OC43 (OC43-bS), and bS2 regions of OC43 (OC43-bS2) and SARS-CoV-2 (bS2). Sequence comparison of the OC43 S2 region to SARS-CoV-2 shows 43% identity. A number of samples from both FC and KD had higher immunoglobulin binding to recombinant bS2 compared to bS, however, comparison of bS to bS2 binding within either FC or KD groups was not statistically significant (Fig. 2A). The interrogation of OC43 antigens showed a number of samples with high normalized binding, which is consistent with the commonality of OC43 infection in this age group and inclusion of humoral immune responses to common hCoV antibodies in pooled IVIG. Although a number of samples were

higher in convalescence, as a whole, these levels were not consistent with seroconversion against any of the antigens tested.

To explore immunoglobulin cross-reactivity to the SARS-CoV-2 S2 regions, we grouped plasma samples (FCs and KD) and the serum KD samples and looked at higher cross-reactive samples by defining high cross-reaction by being >1 standard deviation from the mean (Fig. 2B). For the plasma samples (bS2 sample mean 7.84, standard deviation 14.21, so > 22.05, 12 samples qualified; and OC43 bS2 sample mean 20.76, standard deviation 21.50, so > 42.26, 22 samples qualified). Reactivity of samples with high binding (>1 SD) to SARS-CoV-2 S2 also generally showed high binding to OC43 S2 (*t*-test was not significant), but in comparison to all high cross-reactive to OC43 S2 samples, few bound to the SARS-CoV-2 S2 (p > 0.0001). This non-reciprocal cross-reactivity implies that OC43 reactivity contributes to most of the SARS-CoV-2 cross-reactivty shown in the prepandemic samples. This is consistent with other literature regarding cross reactivity of the S2 region that contributes to Spike reactivity (Wolfel et al., 2020; Nguyen-Contant et al., 2020).
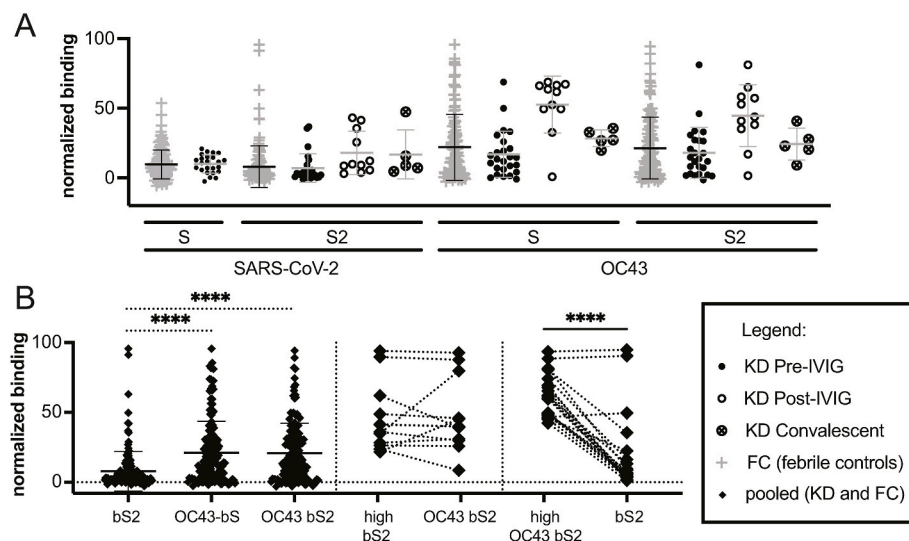
Fig. 2. S2 region targeting of SARS-CoV-2 and OC43 CoVs: Plasma samples from 123 FC were compared to samples from 24 children with KD. For visual comparison, optical density at 450 nm results were normalized by plate by subtracting plate background and comparing to plate positive control set at 100% and means of two experiments were compared. Dotted lines represent percentile binding above background as marked. A) Antigen targeting in FC (grey cross), pre-IVIG KD (black circle), post-IVIG KD (open circle), and convalescent KD (crossed circle). B) Analysis of pooled-sample (diamonds) cross-reactivity based on S2 reactivity (12 samples classified as high bS2 and 22 classified as high OC43 bS2). Statistical comparison was done with Wilcoxon ranked sum tests with p value summaries: * < 0.05 to 0.01, ** 0.009 to 0.001, *** 0.0009 to 0.0001and **** <0.0001. Error bars represent means with standard deviations.

4.3. Additional cohort confirms cross-reactivity post-IVIG in children with KD

To confirm these findings, we obtained serum samples from a cohort of 30 children with KD (KDB cohort), all with complete KD, with serial pre-IVIG, post-IVIG, and convalescent samples (Table 1). Again, administration of IVIG to patients led to increased reactivity in ELISAs. Post IVIG generally showed increased reactivity (Fig. 3) similar to what is shown in Fig. 1, with many samples showing cross-reactive bS protein targeting. Again, All comparisons of sample cohorts pre-IVIG and post-IVIG were significant to varying degrees (chRBD p = 0.0002; hRBD p = 0.0209; bS p = 0.0026; NP p < 0.0001).

On limited sample testing, we once again saw less binding to hTrimS compared to bS protein (see Supplemental Fig. 1). This cohort also showed high specificity of the hRBD produced protein, but one individual (sample 5, cohort #10376) had high reactivity to all commercial reagents, including chRBD.

Consistent with the plasma pediatric samples, many samples in the KD serum cohort showed immunoglobulin binding above background to OC43 antigens (Fig. 4). In comparison of higher SARS-CoV-2 bS2 cross-reactive and lower cross-reactive samples, the majority of those that had high cross S2 reactivity (8) had both high OC43-bS and OC43-bS2 region binding (Fig. 4A). Of those that had lower bS2 reactivity, a minority of samples showed OC43-bS and OC43-bS2 reactivity. This supports that OC43 immunity to the S2 region can also target SARS-CoV-2 S2. Once again, the post-IVIG samples (open circles) saw increases in OC43 binding in both subsets, which was particularly pronounced for the low SARS-CoV-2 cross-reactive subsets (Fig. 4 A and B). These increases in cross-reactions were less pronounced in the convalescent samples suggesting that none of these cases of KD were due to OC43 infection.

To explore a role for potential aberrant coronavirus immune response that would explain aneurysms seen in MIS-C cases, we then correlated specific antigen targeting with presence of aneurysms in the prepandemic KD serum samples. With multiple comparisons using four different antigens, a conservative p value was set at 0.0125 using Bonferroni correction. Pearson correlations of the CoV spike antigens (bS and bS2) were all not significant. The strongest correlations (R = 0.36) were with OC43 bS antigen reactivity and Z score of the LAD (Boston score p = 0.05, Kobayashi score p = 0.054). SARS-CoV-2 bS and Z scores of LAD had negative R values and OC43 bS2 had R = 0.18 with p = 0.33 with both scoring systems. We also analyzed cardiac involvement using Z score cutoffs of both 2 and 2.5 for both Kobayashi (19 and 12 with cardiac involvement) and Boston scoring systems (11 and 8 with cardiac involvement) as detailed in Table 1. Generally, there were no statistically significant differences in antigen targeting between those that had aneurysms and those without aneurysms, although there was a trend toward higher cross-reactivity negatively correlating with aneurysm formation (Fig. 4C).

5. Discussion

As vaccines are being pursued that target the S2 region, knowledge of cross-reactive immunity to this region is needed. Samples from children are ideal to pursue such studies as they are more likely to have had recent exposures to a variety of circulating CoV species. Our study shows non-reciprocal immunity to the S2 region, suggesting that the vaccines targeting the S2 region may have different immune responses dependent on the chosen immunogen. However, immunity to this region does not appear to correlate with any known cardiac issue, supporting the safety of development of such vaccines.

The S2 region of CoVs has a number of traits that are enticing for therapeutic development and vaccine targeting. The S2 region is important in membrane fusion (Anderson et al., 2020; Xia et al., 2019). Amongst the human CoVs, the S2 region has the most sequence overlap (Nguyen-Contant et al., 2020; Beretta et al., 2020), and has been implicated in cross-CoV humoral immunity between the SARS-CoV-2
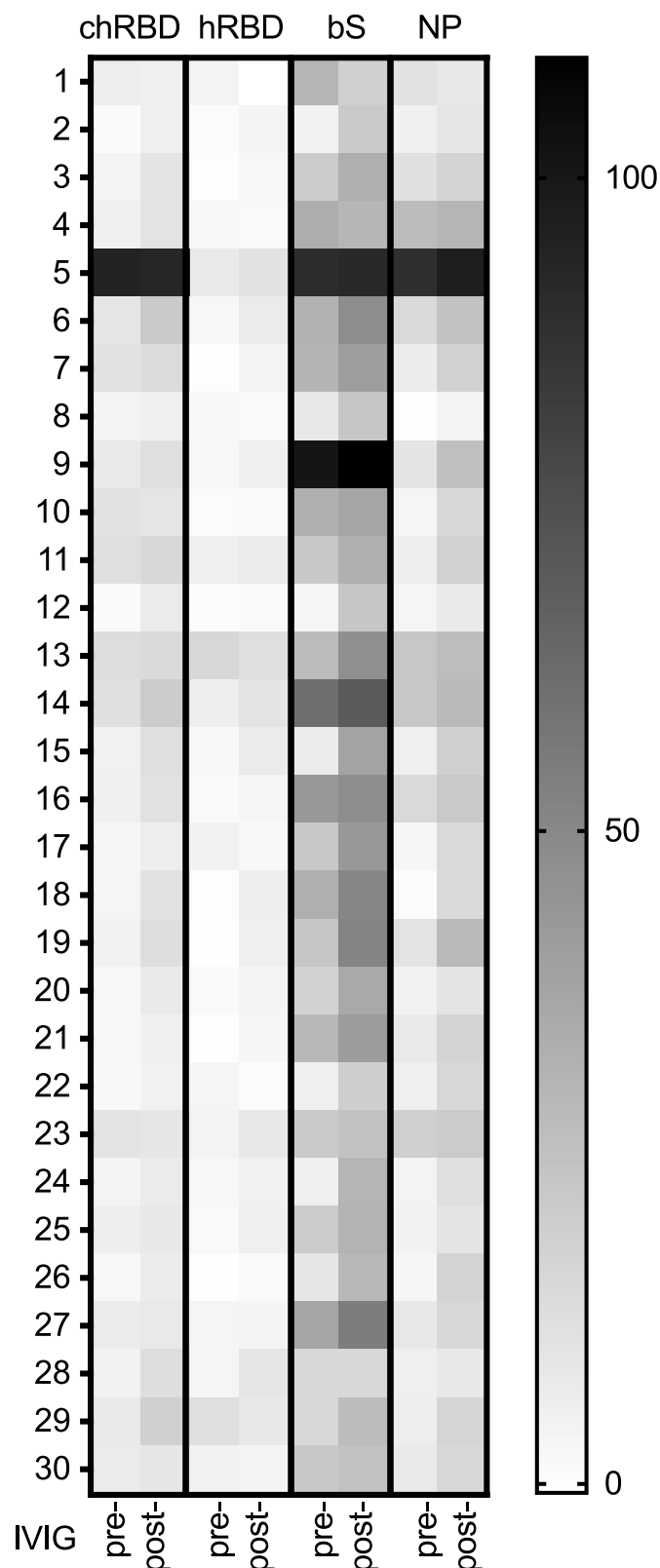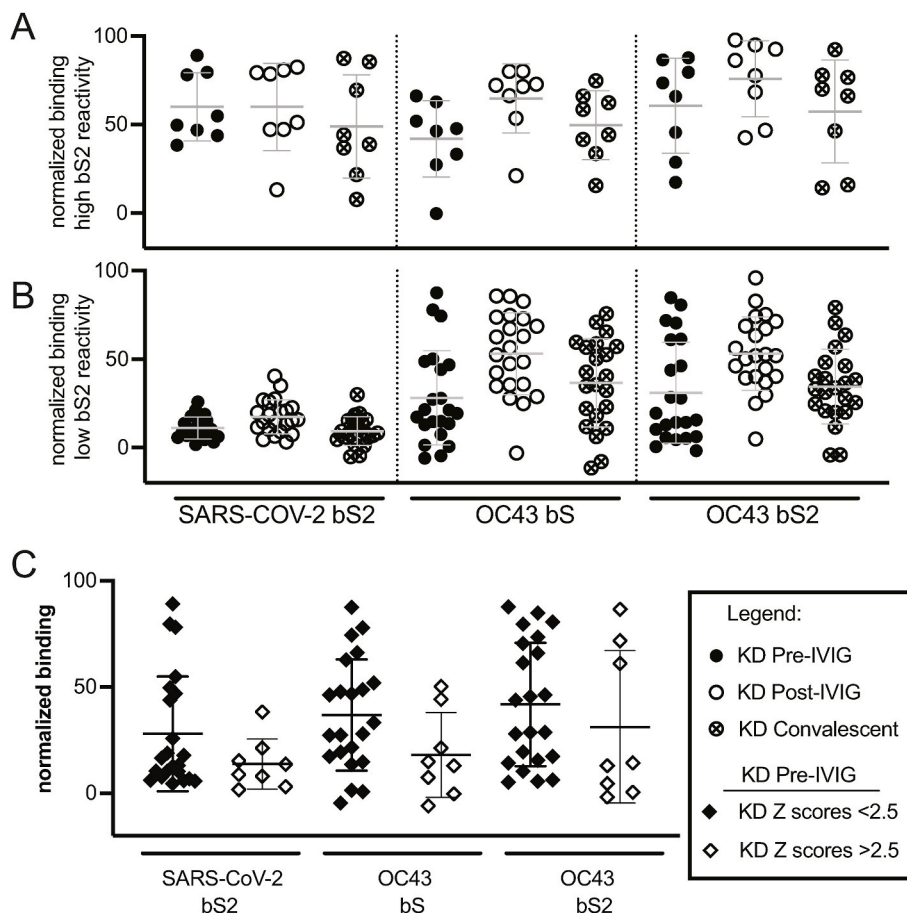

Fig. 3. SARS-CoV-2 antigen targeting of samples in Kawasaki disease cohort pre- and post- IVIG administration. To facilitate intra-antigen comparison, optical density at 450 nm results were normalized by subtracting plate background and comparing positive control set at 100% and means of two experiments were compared. For disparate results, select antigen ELISAs were run in triplicate with outliers of 2-fold difference removed. Heatmap analysis of normalized binding data was done for thirty KDB cohort paired samples pre- and post- IVIG administration with four different antigens.

Fig. 4. Lack of correlation of antigen targeting of SARS-CoV-2 and OC43 with KD aneurysm formation. For visual comparison, optical density at 450 nm results were normalized by plate by subtracting plate background and comparing to plate positive control set at 100% and means of two experiments were compared. For disparate results, select antigen ELISAs were run in triplicate with outliers of 2-fold difference removed. Labels are listed in the legend. A) Antigen targeting of those with high SARS-CoV-2 bS2 cross-reactivity (eight samples), and B) those without low bS2 cross-reactivity (22 samples). C) pre-IVIG samples of those with elevated Z-scores (eight samples) using Boston scoring system were compared to those with non-elevated Z-scores (22 samples).

and other hCoVs, particularly hCoV-OC43 (Anderson et al., 2021b). Peptide based fusion inhibitors, similar to enfuvirtide used for HIV (Monteiro et al., 2021), are being developed for hCoVs. Crystal structures indicate that OC43 derived EK1 can form a stable 6HB structure with both short alpha-hCoV and longer beta-hCoV NHRs, and can interfere in SARS-CoV-2 infection (Xia et al., 2019). Cross-reactive antibody responses to this region are being identified (Shiakolas et al., 2021). Although almost all the neutralizing antibodies of significance target the RBD (Liu et al., 2020; Rogers et al., 2020; Robbiani et al., 2020; Zost et al., 2020), including the majority of the cross-CoV antibodies, there are rare cross-CoV neutralizing antibodies targeting the S2 region (Jennewein et al., 2021).

This study also highlights that the use of IVIG may lead to increased reactivity in immunoglobulin ELISAs. In our study, prepandemic IVIG formulations have minor cross-reactive binding to SARS-CoV-2 antigens, and this likely reflects hCoV cross-immunity as previously noted (Diez et al., 2020). As a majority of the UBKD post-IVIG samples were collected near day ten, we would have expected more significant elevation if this post-IVIG increase was from seroconversion. In both UBKD and KDB cohorts, we did not see evidence of group seroconversion (sustained rise in convalescent samples), although it could not be discounted completely for individual samples.

This study has also has implications for commercial immunoassay development. A number of assays are available to assess clinical post-vaccine S protein targeting. Although most show high specificity, studies have lacked adequate pediatric samples. During the initial description of SARS-CoV-2 human expressed S constructs, specificity of samples was addressed using the alpha-CoV NL63 using solely adult sample (Amanat et al., 2020). There are significant structural differences between the alpha and beta CoVs, with beta-CoVs having shorter HR regions. Tests that relied on fuller regions of S had high specificity, but

these were on cohorts that were solely COVID-19 samples lacking negative controls (National and S.-C.- S.A.E.G., 2020; Simanek et al., 2021; Baro et al., 2021), and rapid test were less specific (Wakita et al., 2021). A recent cohort of infants with PCR confirmed hCoV infections showed targeting to SARS-CoV-2 S2 was increased in samples from infants PCR positive for HKU1 and OC43, although overall they concluded that cross-reactivity was rare (Zar et al., 2021). Secondly, this study shows disparity in antigen reactivity from method of production. Binding to the human expressed trimeric S protein (hTrimS) construct was more specific than monomeric baculovirus (bS) expressed proteins. Likely, monomers present a number of epitopes that are not available on trimeric forms, which is well known for other class I fusion proteins such as HIV (Hicar et al., 2016a, 2016b). Differential his tagging of or difference in glycosylation, a known confounder with baculovirus expression, may also contribute to this difference. This is consistent with prior publications on similar constructs (Amanat et al., 2020). There were also some disparate results on samples with commercially available RBD (chRBD) and expressed RBD. It was intriguing that there was one individual with major disparity between chRBD and hRBD binding (sample 5). On sequence comparison, both of these RBD constructs contained the same S sequence and both are His-tagged, so this differential binding does not have an obvious explanation. Notably, the new variants of concern were not used for any of our assays (Variants of concern, 2021).

6. Conclusions

These findings support that, in relation to cardiac vascular inflammation, vaccines targeting the S2 region appear to be a safe approach, but there is hCOV species disparity in the ability to raise cross-reactive S2 targeted antibodies.

Statement of financial support

CRediT authorship contribution statement

Ajit Monteiro: Writing – original draft, Data curation, Writing – review & editing, drafted the initial manuscript, acquired data, and reviewed and revised the manuscript. **Arthur J. Chang:** Data curation, Writing – review & editing, acquired data, and reviewed and revised the manuscript. **R. Ross Welliver:** Data curation, Writing – review & editing, acquired data, and reviewed and revised the manuscript. **Sarah Baron:** Data curation, Writing – review & editing, acquired data, and reviewed and revised the manuscript. **Mark D. Hicar:** Conceptualization, Data curation, Writing – review & editing, conceptualized and designed the study, coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. All authors declare no competing interests, All authors attest they meet the ICMJE criteria for authorship.

Declaration of competing interest

Acknowledgements:

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virol.2022.08.010.

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