

1 **S protein-reactive IgG and memory B cell production after human SARS-CoV-2 infection**
2 **includes broad reactivity to the S2 subunit**

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13 Running title: Memory B cell response to SARS-CoV-2

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15 Key words: SARS-CoV-2; COVID-19; Memory B cells; IgG antibodies; Spike protein

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18

19 Abstract word count: 248

20 Main text word count: 3659

21

22

23 **ABSTRACT**

24 The high susceptibility of humans to SARS-CoV-2 infection, the cause of COVID-19, reflects the
25 novelty of the virus and limited preexisting B cell immunity. IgG against the SARS-CoV-2 spike
26 (S) protein, which carries the novel receptor binding domain (RBD), is absent or at low levels in
27 unexposed individuals. To better understand the B cell response to SARS-CoV-2 infection, we
28 asked whether virus-reactive memory B cells (MBCs) were present in unexposed subjects and
29 whether MBC generation accompanied virus-specific IgG production in infected subjects. We
30 analyzed sera and PBMCs from non-SARS-CoV-2-exposed healthy donors and COVID-19
31 convalescent subjects. Serum IgG levels specific for SARS-CoV-2 proteins (S, including the RBD
32 and S2 subunit, and nucleocapsid [N]) and non-SARS-CoV-2 proteins were related to
33 measurements of circulating IgG MBCs. Anti-RBD IgG was absent in unexposed subjects. Most
34 unexposed subjects had anti-S2 IgG and a minority had anti-N IgG, but IgG MBCs with these
35 specificities were not detected, perhaps reflecting low frequencies. Convalescent subjects had high
36 levels of IgG against the RBD, S2, and N, together with large populations of RBD- and S2-reactive
37 IgG MBCs. Notably, IgG titers against the S protein of the human coronavirus OC43 in
38 convalescent subjects were higher than in unexposed subjects and correlated strongly with anti-S2
39 titers. Our findings indicate cross-reactive B cell responses against the S2 subunit that might
40 enhance broad coronavirus protection. Importantly, our demonstration of MBC induction by
41 SARS-CoV-2 infection suggests that a durable form of B cell immunity is maintained even if
42 circulating antibody levels wane.

43

44 **IMPORTANCE**

45 Recent rapid worldwide spread of SARS-CoV-2 has established a pandemic of potentially serious
46 disease in the highly susceptible human population. Key questions are whether humans have
47 preexisting immune memory that provides some protection against SARS-CoV-2 and whether
48 SARS-CoV-2 infection generates lasting immune protection against reinfection. Our analysis
49 focused on pre- and post-infection IgG and IgG memory B cells (MBCs) reactive to SARS-CoV-
50 2 proteins. Most importantly, we demonstrate that infection generates both IgG and IgG MBCs
51 against the novel receptor binding domain and the conserved S2 subunit of the SARS-CoV-2 spike
52 protein. Thus, even if antibody levels wane, long-lived MBCs remain to mediate rapid antibody
53 production. Our study also suggests that SARS-CoV-2 infection strengthens preexisting broad
54 coronavirus protection through S2-reactive antibody and MBC formation.

55 INTRODUCTION

56 The betacoronavirus SARS-CoV-2, the causative agent of a respiratory disease termed
57 COVID-19, emerged in China in late 2019 and rapidly spread worldwide (1). A pandemic was
58 declared in March 2020 and global deaths from COVID-19 now exceed 500,000. The rapid
59 increase in cases in many countries has challenged healthcare systems and shutdowns and
60 quarantine measures introduced to slow virus spread have caused major disruptions to society and
61 economies (2). SARS-CoV-2 infection produces a wide spectrum of outcomes. A proportion of
62 infections, likely more than 20%, remain asymptomatic. Most clinical cases develop mild to
63 moderate respiratory symptoms, but up to 20% progress to a more severe disease with extensive
64 pneumonia (3, 4).

65 When SARS-CoV-2 emerged and began to spread, the severity of the threat was primarily
66 attributed to the novelty of the virus to the human immune system and, consequently, a lack of
67 preexisting immune memory to quickly clear virus and limit disease progression. Four types of
68 common cold coronavirus are endemic in humans, the alphacoronaviruses 229E and NL63 and the
69 betacoronaviruses OC43 and HKU1. However, limited relatedness between key structural proteins
70 of these human coronaviruses (HCoVs) and those of SARS-CoV-2 suggested that significant
71 cross-reactive immunity was unlikely (5, 6). Initial studies of non-SARS-CoV-2-exposed
72 individuals found negligible levels of IgG against the SARS-CoV-2 spike (S) protein, the viral
73 attachment protein that binds the receptor angiotensin converting enzyme 2 (ACE2) on host cells
74 to initiate infection (7). More recently, however, studies have provided evidence of SARS-CoV-
75 2-reactive B and T cell memory in unexposed subjects that could confer some protection against
76 SARS-CoV-2 or modulate disease pathogenesis.

77 Sera from non-SARS-CoV-2-exposed individuals have been screened for IgG binding to
78 the S1 and S2 subunits of the SARS-CoV-2 S protein. The membrane-distal S1 subunit contains
79 the receptor binding domain (RBD) for receptor recognition, and the membrane-proximal S2,
80 which has higher homology among coronaviruses than does S1 (6, 8), mediates membrane fusion
81 to release viral RNA into the host cell. In two large cohorts of unexposed subjects, approximately
82 10% had IgG that bound S2, but not S1 or the RBD. Approximately 4% of subjects had IgG against
83 the SARS-CoV-2 nucleocapsid (N) protein, which is highly conserved among coronaviruses (9,
84 10). Although N is an internal viral protein and not a target of neutralizing antibodies (Abs),
85 coronavirus infections typically elicit strong anti-N Ab production (11). The idea that circulating
86 HCoVs elicit IgG that cross-reacts with SARS-CoV-2 is supported by the finding that SARS-CoV-

87 2 infection increases IgG titers against the S proteins of multiple HCoV-229E (12). In T cell studies,
88 CD4⁺ T cells in up to 50% of non-SARS-CoV-2-exposed donors responded to epitopes in S and
89 non-S proteins of SARS-CoV-2 (8, 13). Notably, S-reactive CD4⁺ T cells in unexposed subjects
90 were mostly reactive to the conserved S2 subunit, consistent with cross-reactivity to circulating
91 HCoVs (8). SARS-CoV-2-reactive CD8⁺ T cells were also detected in unexposed donors, but the
92 response was less marked than for CD4⁺ T cells (13).

93 SARS-CoV-2-reactive memory B cells (MBCs) generated in B cell responses to HCoVs
94 are also likely to be present in non-SARS-CoV-2-exposed individuals. Indeed, MBCs might be
95 more important than preexisting cross-reactive Abs as a source of protection against SARS-CoV-
96 2. IgG MBCs are more broadly reactive than Abs generated against the same antigen, they persist
97 after circulating Ab levels wane, and they are readily activated to generate strong Ab responses or
98 seed germinal centers for additional rounds of affinity maturation (14). Concurrent early
99 production of virus-specific IgM and IgG in the response to SARS-CoV-2 infection suggests a
100 response mediated by IgG MBCs as well as naïve B cells (9, 15-17). This picture is supported by
101 identification of B cell subsets with high and low immunoglobulin V gene mutation frequencies
102 during the response to SARS-CoV-2 infection (18). However, little direct analysis of SARS-CoV-
103 2-reactive MBCs in unexposed subjects has been performed.

104 Characterization of MBCs generated and/or expanded by SARS-CoV-2 infection can also
105 provide insights into cross-reactivity between coronaviruses and participation of preexisting
106 MBCs in the response. Wec et al. (19) used cells from a survivor of the 2003 SARS-CoV outbreak
107 as a source of MBCs that bound the S protein of SARS-CoV-2; a comprehensive panel of Abs
108 expressed by the MBCs were cloned and characterized. Notably, most of the highly mutated mAbs
109 bound the S2 subunit of multiple HCoV S proteins, often with higher affinity than to the S2 of
110 SARS-CoV-2. A screening of healthy donors identified low frequencies of MBCs reactive to the
111 S proteins of the 2003 SARS-CoV and SARS-CoV-2 (19). Findings suggest that S2-reactive
112 MBCs generated by HCoVs were activated and expanded by the 2003 SARS-CoV. RBD-binding
113 MBCs sampled in the convalescent phase of SARS-CoV-2 infection expressed Abs with relatively
114 low numbers of V gene mutations, suggesting that this component of the response largely reflected
115 naïve B cell activation by novel epitopes (20).

116 To extend our understanding of the B cell response to SARS-CoV-2 infection, the current
117 study compared Ab and MBC immunity to SARS-CoV-2 in unexposed individuals and individuals
118 in the convalescent phase of infection. In particular, we were interested in the presence of SARS-

119 CoV-2-reactive MBCs in unexposed subjects that could confer some protection against SARS-
120 CoV-2, and formation of MBCs by SARS-CoV-2 infection to provide durable protection against
121 reinfection. Most importantly, we demonstrate that SARS-CoV-2 infection generates both IgG and
122 IgG MBCs reactive to the novel RBD and the conserved S2 subunit of the S protein. Long-lived
123 MBCs are thus likely to be available to mediate rapid protective Ab responses if circulating Ab
124 levels wane and reinfection occurs. Our study also draws attention to preexisting SARS-CoV-2-
125 cross-reactive B cell memory to the S2 subunit in SARS-CoV-2-naïve subjects. We speculate that
126 the strong response to S2 after SARS-CoV-2 infection reflects preexisting S2-reactive MBC
127 activation and strengthens broad coronavirus protection.

128

129 RESULTS

130 **IgG against SARS-CoV-2 proteins in unexposed subjects primarily targets the S2**
131 **subunit of the S protein.** To investigate preexisting B cell immunity to SARS-CoV-2 in
132 unexposed individuals and SARS-CoV-2-reactive B cell immunity generated by infection, we
133 analyzed sera and peripheral blood mononuclear cells (PBMCs) from (i) 21 healthy donors
134 sampled prior to the emergence of SARS-CoV-2, and (ii) 26 non-hospitalized COVID-19
135 convalescent subjects sampled 4-9 weeks after symptom onset. Reactivity was measured against
136 the S (including the RBD and S2 subunit) and N proteins of SARS-CoV-2 and the S proteins of
137 the human alphacoronavirus 229E and betacoronavirus OC43. The H1 influenza virus
138 hemagglutinin and tetanus toxoid (TTd) were included as control antigens that humans are
139 commonly exposed to through infection and vaccination.

140 Serum IgG levels were measured by ELISA. Approximately one-third of non-SARS-CoV-
141 2-exposed subjects in the healthy donor cohort had low levels of serum IgG against the S and N
142 proteins of SARS-CoV-2, likely reflecting cross-reactivity with seasonal HCoVs (Figure 1A).
143 Notably, 86% of unexposed subjects had IgG against the highly conserved S2 subunit of the S
144 protein. It is possible that inherent features of the bulky S reagent used in our analysis reduced
145 binding by anti-S2 Abs. IgG that bound the highly novel RBD was not detected in unexposed
146 subjects. All non-SARS-CoV-2-exposed subjects had IgG against S proteins of the HCoVs 229E
147 and OC43, indicating previous infection, and against the control proteins H1 and TTd (Figures 1C-
148 1F).

149 **S- and N-specific IgG production following SARS-CoV-2 infection includes a strong**
150 **response to the S2 subunit.** Levels of IgG against S, RBD, S2 and N were markedly higher in

151 convalescent subjects than unexposed subjects, indicating strong induction of these Abs by SARS-
152 CoV-2 infection (Figure 1A). In a small number of convalescent subjects, high anti-S IgG titers
153 were associated with low levels of anti-N IgG. Indeed, more than 40% of convalescent subjects
154 had anti-N IgG levels within the range in unexposed subjects, questioning the reliability of using
155 anti-N IgG measurement to identify previous SARS-CoV-2 infection.

156 Notably, serum IgG titers against S2 were consistently higher than against the RBD in
157 convalescent subjects, perhaps reflecting the novelty of the RBD and a response dependent on
158 naive B cell activation (Figure 1B). Interestingly, titers of IgG were higher against the S protein
159 of the HCoV OC43 in convalescent subjects than in unexposed subjects, but this was not the case
160 for the S protein of HCoV 229E (or for the control proteins H1 and TTd) (Figures 1C-1F). The
161 anti-OC43 S IgG titers correlated with those against the SARS-CoV-2 S ($r_s = 0.49$, $P = 0.0109$),
162 RBD ($r_s = 0.57$, $P = 0.0025$), and S2 ($r_s = 0.86$, $P < 0.0001$), indicating a relationship with SARS-
163 CoV-2 infection (Figure 1G). The particularly strong correlation between IgG titers against OC43
164 S and the SARS-CoV-2 S2 suggests a cross-reactive response to the S2 subunit.

165 Since the healthy donor samples in our analysis were collected 6-10 years before the
166 emergence of SARS-CoV-2, we considered the possibility that a recently circulating HCoV could
167 have been responsible for the higher anti-OC43 S IgG titers in the convalescent subjects. To
168 exclude this possibility, we measured anti-OC43 S IgG titers in sera collected from 20 healthcare
169 workers in 2020. The healthcare workers cared for hospitalized SARS-CoV-2 patients, but all were
170 negative for IgG against SARS-CoV-2 S and RBD, consistent with the effectiveness of personal
171 protective equipment and appropriate work practices. OC43 S-reactive IgG levels in healthcare
172 worker sera were similar to those in non-SARS-CoV-2-exposed healthy donor sera and
173 significantly lower than those in sera from convalescent subjects (Figure 1C). Taken together, our
174 results indicate that SARS-CoV-2 infection generates a strong IgG response that cross-reacts with
175 the S2 of human betacoronaviruses.

176 **Strong S-reactive MBC formation following SARS-CoV-2 infection includes**
177 **reactivity to the RBD and S2 subunit.** PBMCs from non-SARS-CoV-2-exposed subjects and
178 convalescent subjects were analyzed for MBCs reactive to SARS-CoV-2 proteins. Circulating
179 antigen-specific IgG MBC populations were measured by in vitro stimulation of MBCs to induce
180 differentiation into Ab-secreting cells (ASCs). Post-stimulation antigen-specific measurement of
181 MBC-derived ASCs (MASCs) by ELISpot assay or MBC-derived polyclonal Abs (MPAbs) by
182 ELISA provided a measure of the precursor MBCs (30). Analysis of MASCs by ELISpot assay

183 was performed against the SARS-CoV-2 S, RBD, and N proteins, and the influenza H1 and TTd.
184 MPABs were measured against antigens used in the ELISpot assay, as well as SARS-CoV-2 S2,
185 and the S proteins of the HCoV OC43 and 229E. Antigen-specific IgG MPAB concentrations
186 correlated strongly with the frequency of IgG MASCs derived from stimulated MBCs (determined
187 for SARS-CoV-2 S and RBD, influenza H1, and TTd, $r_s = 0.89, 0.67, 0.83,$ and $0.95,$ respectively,
188 $P \leq 0.0002$), validating MPAB concentration as a measure of the size of specific MBC populations.

189 The presence of a low level of IgG against the SARS-CoV-2 S, RBD, and N proteins in a
190 proportion of unexposed subjects suggested that IgG MBCs with the same specificity had also
191 been formed. However, these MBCs were not detected, possibly because of very low frequencies
192 in the circulation. In contrast, IgG MBCs reactive to the S proteins of the HCoV OC43 and 229E
193 and the control proteins H1 and TTd were detected in nearly 50% or more of non-SARS-CoV-2-
194 exposed subjects, consistent with the higher levels of serum IgG against these antigens (Figure 2E-
195 2H). As expected, SARS-CoV-2 RBD-reactive MBCs were not detected in unexposed subjects.

196 In marked contrast to non-SARS-CoV-2-exposed subjects, the vast majority of
197 convalescent subjects had circulating IgG MBCs reactive to the SARS-CoV-2 S, RBD, and S2,
198 indicating strong induction by SARS-CoV-2 infection of MBCs reactive to novel and conserved
199 regions of the S protein (Figure 2A). Notably, numbers of IgG MBCs reactive to the S protein of
200 the HCoV OC43 were higher in convalescent subjects than in unexposed subjects (Figure 1E), but
201 there was no difference between the two subject groups in IgG MBCs reactive to the HCoV 229E
202 S protein, influenza H1 or TTd (Figures 2B, 2F-2H). S2-reactive IgG MBC numbers correlated
203 well with IgG MBCs reactive to SARS-CoV-2 S ($r_s = 0.77, P < 0.0001$) and RBD ($r_s = 0.60, P =$
204 0.0012), and S of HCoV OC43 ($r_s = 0.52, P = 0.0059$), but not to S of HCoV 229E ($r_s = -0.13, P$
205 $= 0.53$), influenza H1 ($r_s = 0.13, P = 0.54$), or TTd ($r_s = 0.29, P = 0.15$). The findings of our MBC
206 analysis are consistent with serum IgG measurement and indicate that SARS-CoV-2 infection
207 generates IgG MBCs reactive to the SARS-CoV-2 S2 that cross-react with the S2 of human
208 betacoronaviruses. Interestingly, only a small proportion of the convalescent subjects generated
209 detectable N-reactive IgG MBCs, even though most subjects produced high levels of anti-N IgG
210 in serum (Figures 2C, 2D). It is unclear whether this reflects a real difference between S- and N-
211 reactive MBC formation or an effect of the sampling time. Overall, we demonstrate that SARS-
212 CoV-2 infection induces strong S-reactive MBC formation that would be expected to provide
213 lasting protection against reinfection and potentially broad protection against betacoronaviruses.

214

215 **DISCUSSION**

216 Our goals in this study were to investigate SARS-CoV-2-reactive B cell memory in
217 unexposed subjects that could provide some protection against SARS-CoV-2 infection, and the
218 generation of B cell memory by SARS-CoV-2 infection that could provide lasting protection
219 against re-infection. In particular, we were interested in IgG MBCs, which respond to cognate
220 antigens with rapid, vigorous, and high-affinity Ab production. Importantly, MBCs are long-lived
221 cells that continue to provide strong protection when circulating Ab levels wane. Our approach
222 was to analyze circulating IgG as well as IgG MBCs from the SARS-CoV-2-naïve and SARS-
223 CoV-2-convalescent subject groups. Our key findings are as follows: (i) the presence of IgG
224 reactive to the S2 subunit of SARS-CoV-2 in most unexposed subjects, likely reflecting cross-
225 reactivity to HCoVs, (ii) markedly increased levels of IgG against the SARS-CoV-2 S and N
226 proteins, including reactivity to the RBD and S2 subunit of S, in convalescent subjects, (iii)
227 increased IgG binding to the S protein of the OC43 HCoV, but not 229E HCoV, in convalescent
228 subjects, reflecting greater cross-reactivity between S2 subunits of betacoronaviruses, (iv) strong
229 formation of IgG MBCs reactive with the RBD and S2 subunit of the SARS-CoV-2 S protein in
230 convalescent subjects, and (v) formation of IgG MBCs reactive with the S protein of OC43, but
231 not 229E, in convalescent subjects, consistent with S2 subunit cross-reactivity between
232 betacoronaviruses.

233 Approximately one-third of our cohort of non-SARS-CoV-2-exposed subjects had low
234 levels of IgG against the SARS-CoV-2 S and N proteins. The anti-N IgG likely reflects infection
235 with HCoVs, which have low level (20-30%) homology with the SARS-CoV-2 N protein (10).
236 However, a protective function for anti-N Abs has not been established (21). Notably, 86% of
237 unexposed subjects had IgG against the S2 subunit, reflecting homology with HCoVs, but none
238 had IgG against the highly novel SARS-CoV-2 RBD (6, 8, 22). Abs that target the S2 subunit have
239 been shown to have virus neutralizing activity, raising the possibility that preexisting anti-S2 IgG
240 confers some protection against SARS-CoV-2 (23). The processes that generate anti-S2 IgG are
241 also likely to generate S2-reactive IgG MBCs and these might provide more significant protection
242 than low levels of anti-S2 Abs. However, S2-reactive MBCs (or S-reactive and N-reactive MBCs)
243 were not detected in non-SARS-CoV-2-exposed subjects. Taken together with the identification
244 of S-reactive MBCs in unexposed healthy donors (19), it is likely that S2-reactive MBCs were
245 below the limit of detection in our assays. Most MBCs are resident in lymphoid tissues, except for

246 MBCs against frequently seen immunogenic antigens (for example, the influenza H1 or TTD in
247 this study), and are at very low frequencies in circulation in steady state (24, 25).

248 Anti-RBD, -S, and -N IgG levels were markedly higher in the convalescent subjects than
249 in non-SARS-CoV-2-exposed subjects, indicating strong induction by SARS-CoV-2 infection.
250 Perhaps notably, the majority of convalescent subjects had higher IgG titers against the S2 than
251 against the RBD. This is particularly surprising because of the accessibility of the RBD to B cells
252 and the expected immunodominance over the S2 subunit (26, 27). Our demonstration of strong
253 anti-S2 IgG production is consistent with the activation of a preexisting population of IgG MBCs
254 against the conserved S2 subunit in the absence of MBCs reactive to the novel RBD. However, we
255 cannot exclude inherent differences in the stability or antigenicity of RBD and S2 reagents as an
256 explanation. In convalescent subjects, IgG levels against the S protein of HCoV OC43 (but not
257 229E) were significantly higher than in non-SARS-CoV-2-exposed subjects and correlated
258 strongly with anti-S2 IgG levels. These findings support stronger B cell cross-reactivity between
259 the S2 subunits of SARS-Cov-2 and human betacoronaviruses than alphacoronaviruses (8).

260 Importantly, we demonstrate that SARS-CoV-2 infection generates RBD-reactive and S2-
261 reactive IgG MBCs. Recently, Long et al. (4) found that levels of SARS-CoV-2-reactive Abs,
262 including neutralizing Abs, start to decrease within 8-12 weeks of infection, especially when the
263 infection is asymptomatic. Since MBC populations are maintained for many years, perhaps
264 decades, our findings indicate that MBCs generated by SARS-CoV-2 infection will be available
265 to rapidly generate protective Abs if waning Ab levels allow re-infection to occur (28). Notably,
266 three convalescent subjects in our analysis had undetectable RBD-reactive IgG, but nevertheless
267 had RBD-reactive IgG MBCs. This might reflect MBC production by germinal centers that
268 remained active after recovery from infection (29). The proportion of subjects with MBCs reactive
269 to the HCoVs OC43 and 229E was greater for the convalescent group than the unexposed group,
270 likely reflecting the increase in S2-reactive MBCs in the convalescent group and cross-reactivity
271 with HCoVs. S2-reactive MBC expansion by SARS-CoV-2 infection could enhance protection
272 against a broad range of coronaviruses (23). N-reactive MBC formation in convalescent subjects
273 was less than expected given the large number of subjects with high titers of N-reactive IgG, but
274 additional sampling times are required to confirm this observation.

275 In conclusion, our analysis investigated Ab and MBC immunity to SARS-CoV-2 in
276 unexposed subjects and individuals soon after recovery from SARS-CoV-2 infection. Findings
277 emphasized the novelty of the SARS-CoV-2 S protein RBD in unexposed subjects. However, IgG

278 reactive to the S2 was widespread in unexposed subjects and likely resulted from exposure to
279 HCoV-229E. Although our approach was unable to directly identify S2-reactive MBCs in the
280 unexposed subjects, we suggest that these cells are present and strongly contribute S2-reactive IgG
281 early in the response to SARS-CoV-2 infection. The IgG response in SARS-CoV-2 convalescent
282 subjects was also strong against the RBD and, less consistently, against the N protein. Importantly,
283 SARS-CoV-2 convalescent subjects had generated RBD-reactive and S2-reactive IgG MBCs. The
284 RBD-reactive MBCs are likely to provide strong long-term protection if RBD-reactive neutralizing
285 Ab levels wane and re-infection occurs. Additional studies are required to establish the importance
286 of S2-reactive IgG in providing broad anti-coronavirus activity and the influence of expanded S2-
287 reactive MBC populations on a de novo B cell response to the RBD.

288

289 **MATERIALS AND METHODS**

290 **Study participants and clinical samples.** All study participants were recruited at the
291 University of Rochester Medical Center, Rochester, NY and provided written informed consent
292 prior to inclusion in the studies. The studies were approved by the University of Rochester Human
293 Research Subjects Review Board (protocols 16-0064, 07-0090, and 07-0046) and conducted in
294 accordance with the principles of Good Clinical Practice. A pre-pandemic cohort of 21 healthy
295 donors (median age 48 years, IQR 25-70) were enrolled from 2011-14 (non-SARS-CoV-2-
296 exposed subjects). A cohort of 20 healthcare workers (median age 38 years, IQR 30-52) at Strong
297 Memorial Hospital, Rochester, NY were enrolled in May, 2020. The healthcare workers had not
298 been diagnosed with COVID-19 prior to enrollment. A cohort of 26 non-hospitalized COVID-19
299 convalescent subjects (9 males and 17 females; median age 49 years, IQR 36-63) was enrolled in
300 May, 2020 and consisted of 22 PCR-confirmed patients and 5 non-PCR-confirmed subjects who
301 were contacts of confirmed cases or displayed COVID-19-like symptoms. The convalescent
302 subjects were sampled 4-9 weeks after symptom onset. Symptoms reported (percent of subjects)
303 were fever (67%), cough (74%), sore throat (48%), stuffy/runny nose (56%), difficulty breathing
304 (52%), fatigue (85%), headache (67%), body aches (67%), nausea/vomiting (19%), and
305 diarrhea/loose stool (41%).

306 **Recombinant proteins.** RBD and stabilized ectodomain S protein from SARS-CoV-2
307 (isolate Wuhan-Hu-1) were expressed in-house in HEK293 cells using pCAGGS plasmid
308 constructs kindly provided by Florian Krammer (Icahn School of Medicine at Mount Sinai) (7).
309 Baculovirus-expressed S2 subdomain and HEK293 cell-expressed N protein were obtained from

310 Sino Biological (Chesterbrook, PA) and RayBiotech (Peachtree Corners, GA), respectively.
311 Baculovirus-expressed S proteins from seasonal HCoV OC43 and 229E were obtained from Sino
312 Biological. In-house HEK293 cell-expressed hemagglutinin from egg-derived H1N1
313 A/California/7/2009 and TTd (MilliporeSigma, Burlington, MA) were used as non-coronavirus
314 control proteins.

315 **MBC analysis.** Measurement of antigen-specific MBCs was essentially performed as
316 described previously (30). Briefly, cryopreserved PBMCs were thawed and rested overnight at
317 37°C in complete medium. Rested PBMCs were stimulated for 6 days at 1×10^6 PBMCs/well in
318 24-well plates to induce MBC expansion and differentiation into ASCs. The stimulation cocktail
319 consisted of complete medium supplemented with 1 µg/ml R848 (Sigma, St. Louis, MO), 10 ng/ml
320 IL-2 (Gibco, Gaithersburg, MD), and 25 ng/ml IL-10 (STEMCELL Technologies, Vancouver,
321 Canada). After stimulation, cells were harvested and pelleted by centrifugation. The undiluted
322 supernatant containing Abs secreted by ASCs generated from stimulated MBC precursors
323 (MPAbs) was collected and stored for analysis by ELISA. Supernatants from unstimulated cultures
324 of rested PBMCs were collected to control for Abs produced by preexisting ASCs. Antigen-
325 specific ASCs in the cell pellet (MASCs) were enumerated by ELISpot assay. For each antigen,
326 300,000 stimulated PBMCs were analyzed by ELISpot assay and the limit of MASC detection was
327 set at 8 spots (MASCs)/ 10^6 PBMCs. Based on ELISpot assay results, antigen-specific MBCs in
328 peripheral blood were quantified as antigen-specific IgG MASCs as a proportion of stimulated
329 PBMCs. Antigen-specific IgG concentrations in MPAb samples (after subtraction of Ab
330 concentrations in supernatants from unstimulated PBMC control cultures) were also used as a
331 measure of the relative size of reactive MBC populations.

332 **Enzyme-linked immunosorbent assay (ELISA).** Concentrations of Ag-specific serum
333 Abs and MPAbs were measured by ELISA as previously described (30). Briefly, Nunc MaxiSorp
334 96-well plates (Thermo Fisher, Waltham, MA) were coated overnight with optimized
335 concentrations of antigens. Serially diluted samples were added to blocked plates and incubated
336 for 2 h at room temperature. Alkaline phosphatase conjugated anti-human IgG (clone MT78;
337 Mabtech Stockholm, Sweden) and *p*-nitrophenyl phosphate substrate (Thermo Fisher) were
338 subsequently added to detect bound antigen-specific Abs. Absorbance was read at 405 nm after
339 color development. A weight-based concentration method was used to quantify antigen-specific
340 Ab levels in test samples as described previously (30, 31). Sera from healthy donors and
341 convalescent subjects with high titers for test antigens were used to establish human serum

342 standards. The cutoff for assay positivity was set at approximately 2x the mean OD value for
343 negative wells.

344 **Statistical analyses.** The medians with (q1, q3) were summarized by subject group and
345 compared by the Wilcoxon rank-sum test. Spearman correlation analysis together with
346 corresponding robust regression models was used to assess monotonic associations among Ab
347 responses. Multiple test adjustment was not applied for this explorative study and thus a *P* value
348 < 0.05 was considered significant for all analyses. Statistical analyses were performed using
349 Software SAS 9.4 (SAS Institute Inc, Cary, NC).

350

351 **ACKNOWLEDGMENTS**

352 We thank the staff of the University of Rochester Infectious Disease Research Clinic for
353 subject enrollment and sample collection and BEI Resources for providing some of the reagents
354 used in this study.

355 This project was funded in part with Federal funds from the National Institute of Allergy
356 and Infectious Diseases, National Institutes of Health, Department of Health and Human Services,
357 under CEIRS Contract No. HHSN272201400005C.

358 The authors declare no conflicts of interest.

359

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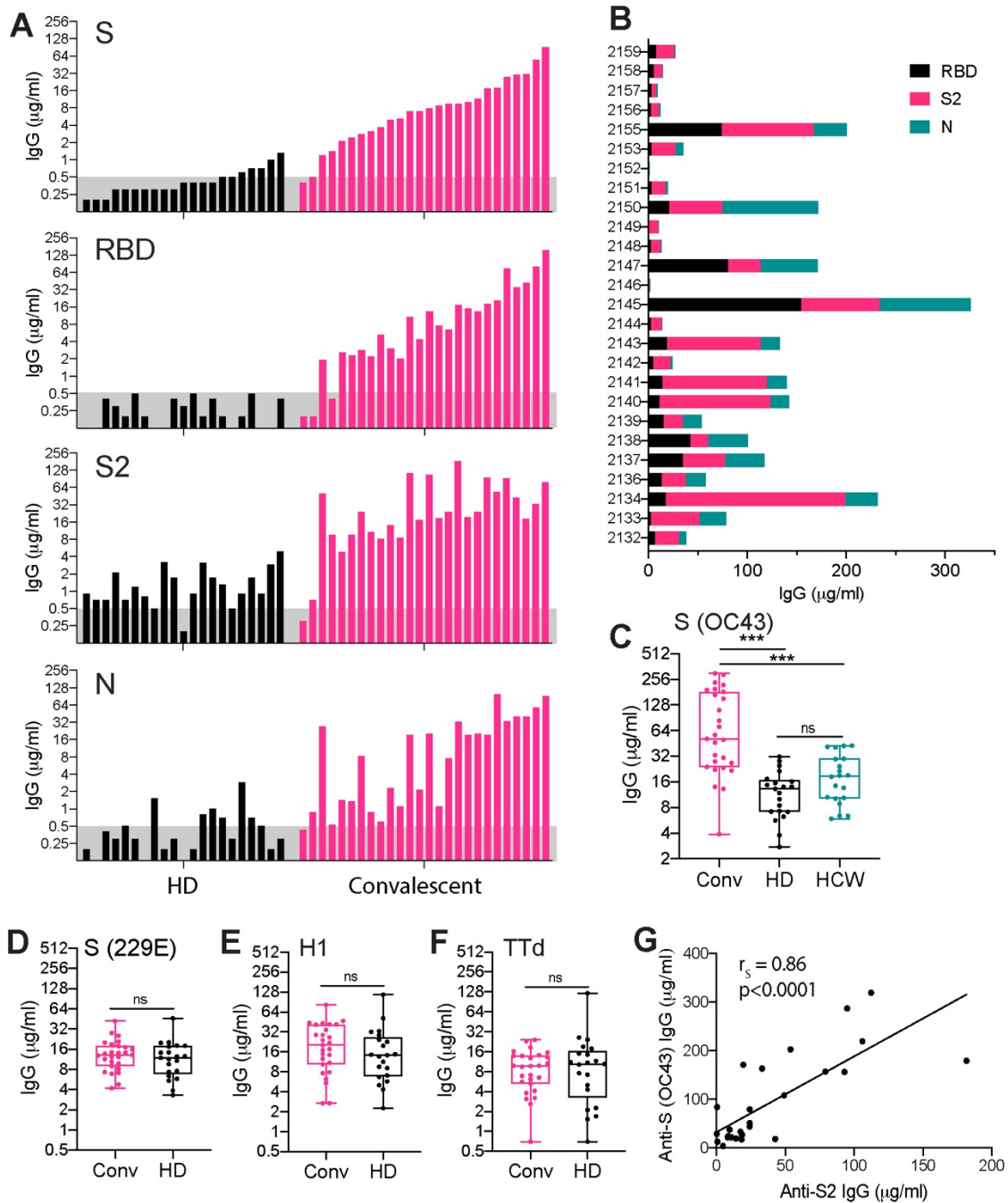
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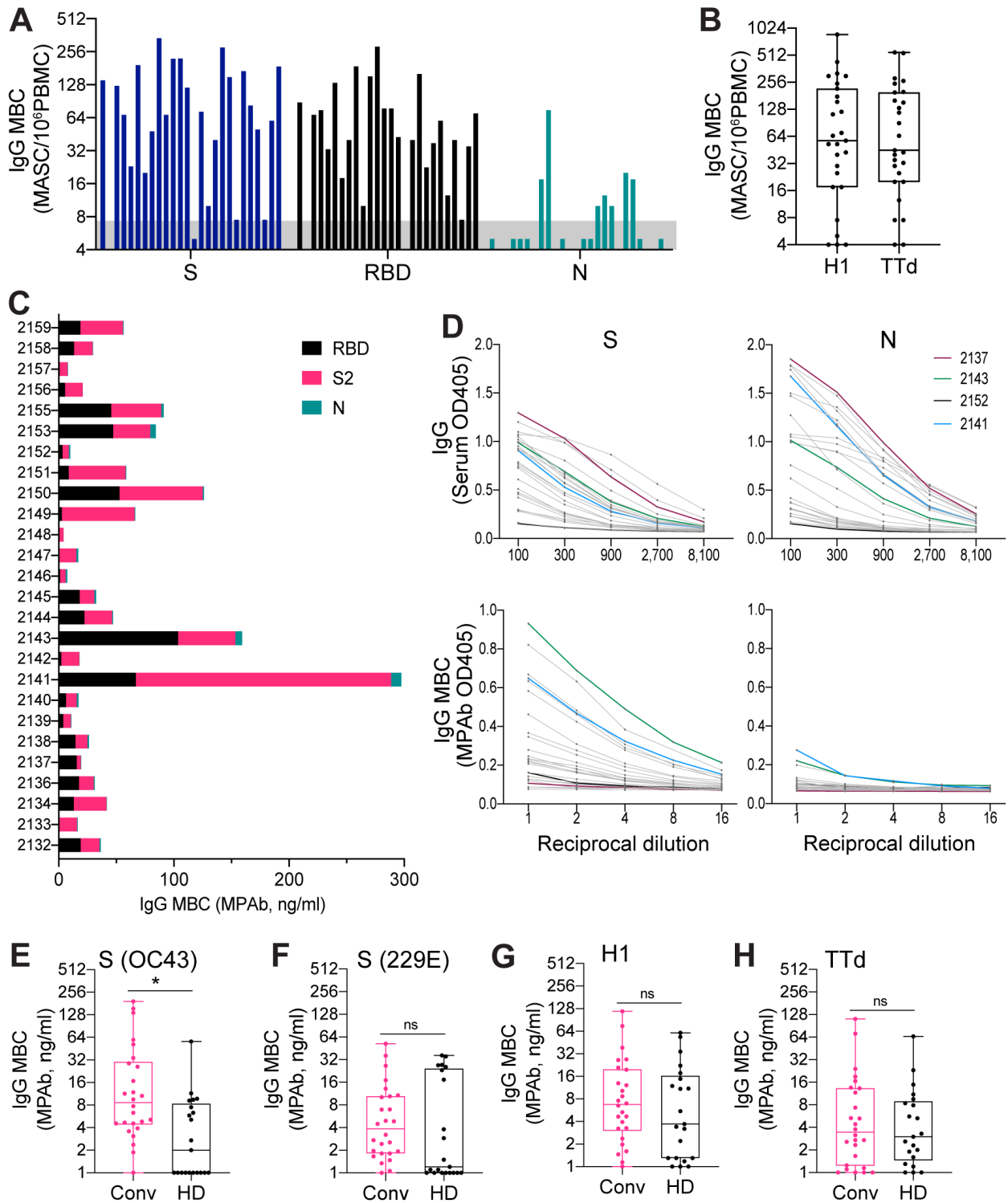
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503 **Figure 1** Serum IgG levels against SARS-CoV-2 and non-SARS-CoV-2 proteins in non-SARS-
 504 CoV-2-exposed and COVID-19 convalescent subjects. Sera were collected from (i) 21 healthy
 505 donors sampled from 2011-14 (HD), (ii) 20 SARS-CoV-2-negative healthcare workers sampled
 506 in 2020 (HCW), and (iii) 26 COVID-19 convalescent subjects sampled 4-9 weeks after symptom
 507 onset (CONV). (A) Serum IgG concentrations measured by ELISA against the SARS-CoV-2 spike
 508 (S), receptor binding domain (RBD), S2 subunit, and nucleocapsid (N). Columns represent

509 individual HD and CONV subjects in order of ascending titers against S. The assigned cutoff for
510 positivity is shown by the shaded bar. (B) Proportions of serum IgG against the SARS-CoV-2
511 RBD, S2, and N for individual CONV subjects. (C) Serum IgG concentrations against the S protein
512 of the HCoV OC43 in CONV, HD, and HCW subjects. (D-F) Serum IgG concentrations against
513 the S protein of the HCoV 229E (D), the influenza virus H1 hemagglutinin (E), and TTd (F) in
514 CONV and HD subjects. (G) Correlation between serum IgG concentrations against the S2 subunit
515 of SARS-CoV-2 and the S protein of the HCoV OC43. Significance (*, $P < 0.05$; **, $P < 0.01$;
516 ***, $P < 0.001$; ns [not significant]) for comparisons of serum IgG concentrations between subject
517 groups was determined by the Wilcoxon rank-sum test. Correlations were tested by Spearman
518 correlation analysis with corresponding robust regression models.
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521 **Figure 2** Analysis of IgG memory B cells (MBCs) reactive to SARS-CoV-2 and non-SARS-CoV-
 522 2 proteins in non-SARS-CoV-2-exposed and COVID-19 convalescent subjects. PBMCs for MBC
 523 analysis were collected from (i) 21 healthy donors sampled from 2011-14 (HD) and (ii) 26
 524 COVID-19 convalescent subjects sampled 4-9 weeks after symptom onset (CONV). PBMCs were
 525 stimulated in vitro to induce MBC differentiation into Ab-secreting cells. Antigen-specific

526 quantitation of MBC-derived Ab (IgG)-secreting cells (MASCs) or MBC-derived polyclonal (IgG)
527 Abs (MPAbs) provided a measure of the abundance of specific IgG MBCs. (A) IgG MBCs reactive
528 to the SARS-CoV-2 spike (S), receptor binding domain (RBD), and nucleocapsid (N) in CONV
529 subjects. MBC numbers were determined by enumeration of IgG MASCs by ELISpot essay after
530 in vitro MBC stimulation. The assigned cutoff for positivity is shown by the shaded bar. (B) IgG
531 MBCs reactive to the influenza virus H1 hemagglutinin and TTd in CONV subjects. MBC
532 numbers were determined by enumeration of IgG MASCs. (C) Proportions of IgG MBCs reactive
533 to the SARS-CoV-2 RBD, S2, and N for individual CONV subjects. (D) Comparison of serum
534 IgG concentrations (upper panels) and IgG MBC numbers (lower panels) reactive to the SARS-
535 CoV-2 S (left-hand side) and N (right-hand side) proteins. Serum IgG was measured by ELISA;
536 IgG MBC numbers were based on ELISA analysis of MPAbs. Dilution curves are shown for
537 individual CONV subjects; curves for 4 subjects are shown in different colors to identify particular
538 response patterns. (E-H) IgG MBCs reactive to the S proteins of HCoV-OC43 (E), and 229E (F),
539 the H1 hemagglutinin (G), and TTd (H) in CONV and HD subjects. IgG MBC numbers were based
540 on ELISA measurements of MPAbs. Significance (*, $P < 0.05$; ns [not significant]) for
541 comparisons of IgG MBC numbers between subject groups was determined by the Wilcoxon rank-
542 sum test.

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