1 **Hybrid immunity elicits potent cross-variant ADCC against SARS-CoV-2 through a**

2 **combination of anti-S1 and S2 antibodies**

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4 **Running Title: Anti-S1 and S2 antibody responses support robust ADCC**

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Abstract

Antibodies capable of neutralising SARS-CoV-2 have been well studied, but the Fc receptor-dependent antibody activities that also significantly impact the course of infection have not been studied in such depth. SARS-CoV-2 infection induces antibody-dependent NK cell responses targeting multiple antigens, however, as most vaccines induce only anti-spike antibodies, we investigated spike-specific antibody-dependent cellular cytotoxicity (ADCC). Vaccination produced antibodies that only weakly induced ADCC, however, antibodies from individuals who were infected prior to vaccination ('hybrid' immunity) elicited much stronger anti-spike ADCC. Quantitative and qualitative aspects of humoral immunity contributed to this capability, with infection skewing IgG antibody production towards S2, vaccination skewing towards S1 and hybrid immunity evoking strong responses against both domains. The capacity for hybrid immunity to provide superior spike-directed ADCC was associated with selectively increased antibody responses against epitopes within both S1 and S2. Antibodies targeting both spike domains were important for strong antibody-dependent NK cell activation, with three regions of antibody reactivity outside the receptor-binding domain (RBD) corresponding with potent anti-spike ADCC. Consequently, ADCC induced by hybrid immunity with ancestral antigen was conserved against variants containing neutralisation escape mutations in the RBD [Delta and Omicron (BA.1)]. Induction of antibodies recognizing a broad range of spike epitopes and eliciting strong and durable ADCC may partially explain why hybrid immunity provides superior protection against infection and disease than vaccination alone, and demonstrates that spike-only subunit vaccines would benefit from strategies to induce a combination of S1- and S2-specific antibody responses.

Significance

Neutralising antibodies prevent the entry of cell-free virus, however, antibodies that promote Fc-dependent activities such as ADCC are critical to control cell-associated virus. Although current SARS-CoV-2 vaccines induce potent neutralising antibodies, they fail to induce robust ADCC. Our demonstration that hybrid immunity induces superior ADCC with pan-variant activity may partially explain why hybrid immunity offers enhanced protection against reinfection. It also highlights that vaccine strategies based on expression of the spike subunit alone should not focus solely on inducing antibody responses targeting the receptor binding domain.

Key words: SARS-CoV-2, COVID-19, antibodies, NK cell, ADCC

Introduction

Humoral immunity against SARS-CoV-2 spike (S) protein is induced by infection and by vaccination with any of the predominant COVID-19 vaccines used worldwide, most of which encode S as a single antigen (1-3). Anti-S antibodies (Ab) target multiple regions within the protein, but the major focus has been on those that neutralise cell-free virions. These primarily bind within the receptor binding domain (RBD) or in some cases the N-terminal domain (NTD), both of which are found in the S1 domain of the protein. Neutralising Abs block or prevent binding between SARS-CoV-2 and the entry receptor angiotensin converting enzyme-2 (ACE-2) or prevent post-binding events required for virus entry (4, 5). They are thought to be crucial for reducing transmission of SARS-CoV-2, and thus, are a key measure for predicting COVID-19 vaccine efficacy (6).

Despite their clear importance, neutralising Abs have recognised limitations. The number of neutralising epitopes is limited, resulting in rapid selection of SARS-CoV-2 variants with mutations that weaken Ab binding to key neutralising sites (7, 8). After approximately 3 years of evolution in the human population, SARS-CoV-2 variants of concern have largely escaped the neutralising activity of Abs induced by the ancestral S antigen and are continually evolving to evade Abs induced by infection with more recent variants. As a result, the efficacy of vaccines at preventing infection is already reduced within months of their introduction. Once infection occurs, SARS-CoV-2 can undergo direct cell-cell transmission, which further undermines the efficacy of neutralising Ab (9).

To counteract cell-cell virus spread, Abs are required which, rather than neutralising cell-free virions, recognise viral antigens on the surface of infected cells (10). These recruit effector cells such as natural killer (NK) cells to kill infected cells through antibody-dependent cellular cytotoxicity (ADCC), thereby controlling cell-associated virus. Infection with SARS-CoV-2 readily induces Ab capable of supporting ADCC (11) and ADCC is a key determinant of immunological control in animal challenge models (12-21). We and others have shown that Ab capable of ADCC are effective at preventing disease in animals even in the complete absence of neutralising activity (18, 22). Thus, inducing and maximising this activity through vaccination is highly desirable.

In addition to being a target for neutralising Abs, SARS-CoV-2 S is also expressed on the infected cell surface, where it is efficiently bound by Abs (11). Thus, S has the potential to be an effective target for Fc receptor (FcR)-mediated NK cell activation, providing a single vaccine antigen capable of inducing Ab activity targeting both cell-free and cell-associated virus. Although studies have shown that vaccination

104 induces anti-S Abs capable of activating NK cells when tested against purified protein (23-25) or

105 transfected cells (26-31), our previous study revealed that, when tested against live virus, effective ADCC

106 was dominated by Abs targeting nucleocapsid (N), membrane (M), and ORF3a and individuals who had

107 only anti-S Abs (i.e. vaccinees with no infection history) demonstrated weak ADCC (11).

108 Subsequent studies have made it clear that infection with SARS-CoV-2 prior to vaccination with a S-

109 encoding vaccine ('hybrid' immunity) offers superior protection compared to vaccination or infection

110 alone (32-35). To explore Fc-dependent mechanisms that may contribute to this protection, we examined

111 quantitative and qualitative aspects of S-induced humoral immunity and its association with ADCC

112 potency in individuals who had recovered from infection, who had been vaccinated, or both.

Results

Hybrid immunity elicits robust ADCC against S-transduced cells

115 As all donors in this study were vaccinated with SARS-CoV-2 S-encoding vaccines, we isolated S-specific

116 ADCC from responses targeting other SARS-CoV-2 proteins by testing Abs for their capacity to mediate

117 lysis of SARS-CoV-2 S-expressing target cells. A series of plasma dilutions were performed initially to

118 establish optimal levels on a subset of samples and plasma was diluted 1:1000 for subsequent

119 experiments. This dilution distinguished ADCC from no ADCC for vaccinee and post-infection samples and

120 discriminated ADCC levels from hybrid immunity across a wide range without plateauing.

121 There was minimal background ADCC against parental (non-transduced) MRC-5 elicited by SARS-CoV-2

122 seropositive plasma and against Wuhan-Hu-1 S-expressing MRC-5 (Wu-S-MRC-5) cells by pre-pandemic

123 plasma (Figure 1a). Consistent with previous data (11), plasma from participants recovered from SARS-

124 CoV-2 infection mediated weak S-specific ADCC (mean 7.0% lysis) with Abs from only 13/31 individuals

125 inducing > 10% lysis (Figure 1b). Responses were weaker amongst those who were vaccinated and not

126 previously infected, with only one participant mediating ADCC > 10% after one vaccination (Figure 1b). A

127 second vaccination increased responses with 10/40 individuals demonstrating killing above 10% specific

128 lysis (Figure 1b). Nevertheless S-specific ADCC remained low overall, comparable overall to that of

129 participants with infection-induced immunity (mean 7.7% lysis; Figure 1b). In contrast, hybrid immunity

130 substantially enhanced ADCC (mean 31.3% lysis) with the absolute number of participants mediating >

131 10% lysis increasing to 29/31 after a single vaccination (Figure 1b). There was no further increase to ADCC

132 within the hybrid group upon second vaccination, however, one additional participant (30/31) mediated

133 > 10% ADCC (Figure 1b).

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Hybrid immunity induces significant S-specific ADCC against infected cells

134 Although cells transfected or transduced to express S provide a platform to isolate S-specific ADCC, Ab-

136 dependent NK cell responses in the context of virus infection can differ significantly from that with cells

137 overexpressing surface protein (11). As ^{51}Cr release assays are incompatible with BSL3 conditions, we

138 assessed Ab-dependent NK cell activation (ADNKA) against SARS-CoV-2 infected cells by measuring NK cell

139 degranulation (CD107a) as a surrogate for ADCC.

140 During infection, ADNKA is dominated by Ab targeting antigens other than S, obscuring increases in S-

141 specific ADNKA attributed to vaccination (11). To circumvent this issue, we focused on individuals with

142 asymptomatic or mild infection (and, therefore, comparatively weak pre-vaccine ADNKA responses)

143 followed by vaccination with a S subunit vaccine, and tested sera over a range of dilutions. This allowed

144 us to select a dilution at which infection-induced anti-N/M/ORF3a responses had faded, but where a

145 significant boost to S-specific ADNKA could be measured following vaccination (Figure 1c) across multiple

146 donors (Figure 1d). Although a second vaccine dose enhanced the abundance of ADNKA-capable Ab in

147 some donors, such that reactivity was maintained at higher dilutions (Figure 1c), it did not alter maximal

148 levels of ADNKA (Figure 1c, d).

149 Therefore, anti-S Abs can mediate S-specific Ab-dependent NK cell activity against transduced or infected

150 cells. However, these Abs are not efficiently induced by natural infection or vaccination – a hybrid

151 combination is required.

Neutralisation and ADNKA engage different Ab populations

153 We next used infected cells to compare S-specific ADNKA responses mediated by hybrid immunity induced

154 by mild or severe infection to determine whether S-specific ADNKA provided by hybrid immunity

155 approached the level of ADNKA induced by the sum of Ab targeting all SARS-CoV-2 cell-surface proteins

156 (*i.e.* including anti-N/M/ORF3a; Figure 2a) (11). Individuals were further stratified by the vaccine they

157 received [adenovirus (AstraZeneca ChAdOx1-S) or mRNA (Pfizer-BioNTech)]. Vaccination alone induced

158 weak ADNKA, irrespective of vaccine platform (Figure 2a, b). In contrast, hybrid immunity boosted S-

159 specific ADNKA to levels comparable to the potent multi-antigen ADNKA response of individuals who had

160 recovered from mild infection, but not to the extent of those recovered from severe infection (Figure 2b).

161 This pattern was in sharp contrast to the neutralising Ab response, where vaccination induced responses

162 comparable to mild infection, and hybrid immunity gave responses comparable to severe infection (Figure

163 2c).

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164   To investigate further, we considered the correlation between neutralising and ADNKA Ab titres against

165   live virus. Comparisons amongst vaccinated individuals in which ADNKA was primarily driven by anti-S

166   responses demonstrated a positive correlation between neutralisation and ADNKA, along with a clear

167   hierarchy of responses. Vaccination with adenovirus (AstraZeneca ChAdOx1-S) demonstrated the weakest

168   neutralisation and ADNKA activity, mRNA (Pfizer-BioNTech) vaccination induced better ADNKA and

169   neutralisation than adenovirus vaccination, and both vaccination regimes were inferior to neutralisation

170   and ADNKA induced by hybrid immunity (Figure 2d). Although neutralisation and ADNKA showed positive

171   correlations in vaccinees and infected individuals (11), the relationship between the two activities was

172   markedly different. For any given level of neutralisation, infection showed superior levels of ADNKA

173   compared to vaccination (Figure 2e). Thus, in comparison to infection, S-based vaccines are effective at

174   inducing neutralising Abs and less so at inducing ADNKA, consistent with the presence of Abs against

175   multiple different ADCC targets following infection.

176   **Superior ADCC from hybrid immunity is not strictly explained by Ab abundance**

177   To test whether differences in ADCC between hybrid and vaccine-induced immunity were due in part to

178   IgG abundance, we compared ADCC with measurements of total IgG targeting full length S (FLS) and

179   individual S1 and S2 subunits. Comparisons were also carried out on IgG_3 subclass Ab; although IgG_1 is

180   most abundant, IgG_3 supports more potent ADCC (36, 37).

181   ADCC positively correlated with amounts of anti-S1 and S2 IgG (Figure 3a) and IgG_3 (Figure 3b) Abs, despite

182   anti-S1 IgG_3 not being abundant in those recovered from infection (Figure 3b). As the hybrid group

183   demonstrated more robust ADCC after first vaccination and there were no significant increases in the

184   levels of Abs or ADCC after second vaccination (see below), we focused on responses elicited from the

185   hybrid group after one vaccine to contrast with the vaccinees post second vaccination. In both the hybrid

186   cohort (PV1) and the vaccine group (PV2), ADCC correlated with increased anti-FLS IgG Ab levels (Figure

187   3c). This trend persisted when we compared anti-S1 IgG (Figure 3d) and anti-S2 IgG (Figure 3e) Ab levels

188   with ADCC. There was also a significant correlation between levels of anti-FLS IgG_3 Ab and ADCC in both

189   the hybrid and vaccine cohorts (Figure 3f), although this correlation was lost for the hybrid group when

190   comparing reactivity against individual S1 and S2 subunits of SARS-CoV-2 S (Figure 3g, h). This may reflect

191   that the lower abundance of IgG_3 results in IgG_1 having a greater influence on total ADCC activity.

192   Despite these strong correlations between Ab levels and ADCC *within* cohorts, there were significant

193   differences *between* the cohorts, with Abs from vaccinees eliciting much weaker ADCC for a given level of

194   Ab compared to their hybrid counterparts. Thus, although Ab levels influence ADCC activity, Ab abundance

195   alone does not explain the superior ADCC induced by hybrid immunity.

Vaccination, infection, and hybrid immunity induce distinct antibody responses to S1 and S2

197   Since strong ADCC induced by hybrid immunity reflects the quality of immune response rather than simply

198   abundance of IgG, we investigated the specificity of anti-S Abs following vaccination or hybrid immunity

199   in more detail. Circulating IgG Abs against FLS and the individual S1 and S2 domains were measured by

200   ELISA after infection, and after first and second vaccinations. Anti-FLS IgG Abs were detected (OD > 0.1)

201   from 30/31 of the participants included in the hybrid cohort, and 38/40 of the vaccinated individuals

202   (Figure 4a). Within the hybrid cohort, levels of anti-FLS Abs rose significantly after first vaccination, but

203   there was no further increase upon second vaccination (Figure 4a). In contrast, Ab levels for the vaccinee

204   cohort increased significantly following the second vaccination but remained relatively low (Figure 4a).

205   Infection generated IgG Abs against both S1 and S2 domains, however, anti-S2 Ab responses were

206   favoured over anti-S1 (Figure 4b). Vaccination after infection increased anti-S1 IgG Ab levels but had no

207   significant impact on the levels of anti-S2 IgG Abs, indicating preferential boosting of anti-S1 Ab (Figure

208   4b). Similar skewing towards anti-S1 responses following vaccination was also apparent amongst those

209   vaccinated with no prior infection, as substantially higher levels of anti-S1 IgG Abs compared with anti-S2

210   IgG Abs arose after both first and second vaccinations (Figure 4c). Thus, the anti-S IgG Ab response differs

211   following infection or vaccination, with vaccination selectively inducing anti-S1 IgG Abs, while infection-

212   induced immunity promoted higher levels of anti-S2 IgG Abs.

Infection history dictates anti-S2 IgG$_3$ antibody responses

214   In addition to measuring the proportion of total IgG targeting S, we assessed the prevalence of IgG$_3$ Abs

215   in persons with hybrid or vaccine-induced immunity. Anti-S1 IgG$_3$ Ab levels after infection were generally

216   low, with only 4/31 participants having OD > 0.1 (Figure 4d), but levels increased after first vaccination

217   with anti-S1 IgG$_3$ Abs detected in 11/31 participants (Figure 4d). In contrast, anti-S2 IgG$_3$ Ab levels were

218   more substantial following infection, with 21/31 of participants having detectable anti-S2 IgG$_3$ Abs (Figure

219   4d) and vaccination further improved anti-S2 IgG$_3$ Ab levels (Figure 4d). With hybrid immunity, levels of

220   anti-S2 IgG$_3$ Abs were significantly higher than anti-S1 IgG$_3$ Abs and remained stable up to ~5 months.

221   Vaccination alone resulted in 28/40 individuals producing anti-S1 IgG$_3$ Abs after first vaccination and 31/40

222   after second vaccination (Figure 4e). One vaccination induced low amounts of anti-S2 IgG$_3$ Abs in 16/40

223   persons that improved upon second vaccination with 30/40 participants having detectable anti-S2 IgG$_3$

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224   Abs (Figure 4e). Compared with hybrid immunity, levels of anti-S1 IgG_3 Abs outweighed anti-S2 IgG_3

225   following a single vaccination, with levels similar following two vaccinations (Figure 4e). Therefore, for

226   both total IgG and IgG_3, infection-induced immunity favoured anti-S2 Ab production, whereas vaccination

227   induced more robust anti-S1 Ab levels (Figure 4f, g). The strongest IgG Ab responses against both S1 and

228   S2 were seen following hybrid immunity.

229   **ADCC potency depends on antibodies reactive against both S1 and S2**

230   To determine whether Abs targeting either or both of the S1 and S2 domains were responsible for potent

231   S-specific ADCC following hybrid immunity, we selectively depleted Abs that target different S domains

232   and assessed ADNKA against infected cells. ADNKA induced by vaccinee serum was too low to observe

233   clear effects following depletion so we focussed solely on donors with hybrid immunity. Using two donors,

234   we depleted Abs targeting the entire S trimer, the S1 domain, the S2 domain, or specific NTD or RBD

235   domains found within S1. Serums were pre-diluted such that the starting dilution was sufficient to have

236   diluted out Abs targeting antigens other than S (Figure 1c). Although depleting Abs targeting NTD or RBD

237   alone led to only minor reductions in ADNKA, complete S1 or S2 subunit Ab depletion led to significant

238   reductions in both donors (Figure 5a, b), indicating that a combination of Abs targeting S1 and S2 is

239   required for robust ADNKA.

240   Depleting Abs targeting NTD or RBD yielded smaller reductions in ADNKA than depleting Abs targeting the

241   entire S1 domain. The reagents used for NTD and RBD depletion targeted amino acids 16-318 and 319-

242   541, respectively, while the S1 depletion targeted amino acids 16-685. As the S1 depletion potentially

243   removed Abs that targeted 542-685, which were not depleted by RBD or NTD depletion, we repeated this

244   experiment and included a double depletion of both NTD and RBD (Figure 5c, d). The RBD/NTD double

245   depletion closely mirrored S1 depletion. Combined data from five donors indicated that potent ADNKA

246   induced by hybrid immunity was dependent on the presence of Abs reactive to both the S1 <u>and</u> S2 regions,

247   as depletion of either region led to a significant loss in ADNKA (Figure 5e).

248   **Antibody reactivity against three determinants along S correlates with potent ADCC**

249   Having determined that Abs against both S1 and S2 subunits were important for eliciting strong S-specific

250   ADCC, we investigated the fine specificity of Ab responses arising from hybrid immunity by ELISA-based

251   peptide scanning. 181 overlapping peptides were coated onto ELISA plates in sequential pairs and IgG Ab

252   reactivity was measured. A comparison of Abs reactive to linear regions contained in both S1 and S2

253   produced after infection (Figure 6a, blue line) and subsequent vaccination (Figure 6a, orange line)

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254   revealed differential patterns and robustness of Ab reactivity. A heatmap illustrating changing patterns of

255   linear Ab epitope reactivity along S before and after vaccination is depicted for 7 participants in the hybrid

256   group (Figure 6b).

257   Samples obtained after infection alone had Abs recognising fewer S1 and S2 epitopes than matched

258   samples following vaccination (Figure 6b), with Ab reactivity against three distinct regions along S

259   particularly enriched amongst the hybrid samples (Figure 6b). These three areas of reactivity are

260   contained within the (i) C-terminal domain (CTD) 1 and (ii) CTD2 of S1 and (iii) a region in S2 immediately

261   upstream of the heptad repeat 2 (HR2) sequence in the connector domain (CD; Figure 6b). Besides the

262   early arising and common D614G mutation, these regions showed no genetic variation between Delta or

263   Omicron variants (Figure 6b).

264   To illustrate potential associations between ADCC and Ab reactivity against these three regions, we used

265   data collected from two persons with infection-induced immunity and subsequent vaccination to depict

266   the relationship between ADCC and the magnitude of anti-IgG Ab found in CTD1/2 and CD. In both cases,

267   increasing anti-IgG Ab levels specific for each of the three regions paralleled robust increases in ADCC

268   (Figure 6c). Further, there were significant associations between ADCC and both the number of ADCC

269   determinants to which a participant demonstrated IgG Ab reactivity (Figure 6d) and the cumulative OD

270   from the three distinct regions (Figure 6e). Thus, epitope specificity plays a key role in S-specific killing of

271   SARS-CoV-2-infected cells.

Hybrid immunity broadly mediates ADCC across variant strains

273   Virus evolution in human populations results in selection of viral variants mutated at key residues for

274   neutralising Ab binding – primarily within the RBD, but also within the NTD (7, 8). However, ADCC-

275   susceptible determinants are distributed much more broadly across S (Figure 5) and ADCC determinants

276   revealed by peptide scanning do not overlap with mutations in recent virus variants such as Delta and

277   Omicron (Figure 6b). This suggests ADCC induced by hybrid immunity may be better preserved than

278   neutralisation across variant strains. To assess S-specific Ab-dependent responses against variant strains,

279   MRC-5 cells were transduced to express Delta or Omicron S at levels equivalent to Wu-S-MRC-5 (Figure

280   7a). When used in ADCC assays, Abs produced after hybrid infection elicited comparable levels of ADCC

281   against target cells expressing Wuhan-Hu-1 and Delta S (Figure 7b). Although ADCC was reduced against

282   Omicron S-expressing cells, the relative reduction was mild (12.9% decrease), and 5/16 participants

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283   mediated equivalent levels of ADCC against cells expressing Wuhan-Hu-1, Delta and Omicron S (Figure

284   7b).

285   A subset of vaccinees mediating detectable ADCC against Wu-S-MRC-5 were similarly tested. Here, the

286   decline in ADCC against cells expressing Omicron S was more pronounced with a 35.5% relative reduction

287   (Figure 7c). Ab from only 1/14 vaccinees mediated comparable levels of ADCC against wild type and

288   variant strains (Figure 7c), consistent with Ab in these donors being focussed on the S1 domain, which is

289   more heavily mutated in these virus variants.

290   To ensure that these results were comparable to an infection setting, we assessed neutralisation and

291   ADCC against replicating virus using either ancestral Wuhan-Hu-1 or Omicron strains. Consistent with

292   published data, we observed that Omicron was more resistant to neutralisation following vaccination, and

293   despite this decrease in neutralisation being less severe following hybrid immunity compared with

294   vaccination, a significant loss was observed (Figure 7d). In contrast, and in agreement with data obtained

295   using cells overexpressing S, we measured only a small decline in ADNKA against Omicron-infected cells

296   (Figure 7e). Thus, by targeting a broader range of epitopes, including those more conserved across

297   variants, ADCC resists virus escape due to virus mutation more effectively than neutralisation.

Discussion

299   Strong and durable immune responses are desirable to limit SARS-CoV-2 transmission and control severity

300   of infection. Neutralising Ab activity has dominated as a surrogate measure of protection, however,

301   protection against severe illness without robust neutralisation suggests that other aspects of immunity,

302   including T cells and NK cells, play a role (38). The ability of T cells and NK cells to limit illness by eliminating

303   infected host cells once infection does occur underlies the importance of considering their recruitment in

304   vaccination strategies, especially if their activity is better conserved across variants than Ab neutralisation

305   (39, 40).

306   Associations between ADCC and viral control in animal models (12-22) indicate that it is an important

307   component of immunological protection. Infection with SARS-CoV-2 induces Abs against N/M/ORF3a that

308   dominate NK cell activation by infected cells (11), however, in the absence of vaccines encoding

309   alternative ADCC antigens, it is important to understand the capacity for S-specific ADCC. Infection or

310   vaccination alone was insufficient to induce potent S-directed ADCC, whether assessed by direct killing of

311   S-expressing cells or NK cell activation against SARS-CoV-2 infected cells. However, the strong ADCC

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312 observed with hybrid immunity indicates that it is possible to generate robust and lasting ADCC through

313 S-targeted Ab responses.

314 Previous analysis of hybrid immunity in the context of neutralising Abs demonstrated that compared to

315 vaccination alone, it results in more memory B-cells and circulating Abs, the latter of which was affirmed

316 in our study (41, 42). This increase in circulating IgG enhances neutralisation of more immune-evasive

317 virus variants (41, 43, 44), while at the clonal level, it also reflects selection of Abs with higher neutralising

318 potency (45). In the case of ADCC, neither increased abundance, nor differences in IgG subclass could

319 explain superior hybrid immunity-induced ADCC. Instead, qualitative aspects of the Ab profile played a

320 more significant role. Robust levels of Abs targeting numerous epitopes in both S1 and S2 were a

321 consistent feature of hybrid immunity.

322 The S1 domain of spike contains the NTD and RBD domains, which are required for receptor binding and

323 are dominant targets for neutralising Abs. As a result, vaccine development has focussed heavily on

324 inducing S1-targeted Abs, including the testing of RBD subunit vaccines (46-50). However, due to

325 mutations selected in S1 that tend to reduce neutralisation, there is increasing interest in neutralising Abs

326 targeting the more conserved S2 domain (51). Studies in mice demonstrated that S2-specific vaccination

327 effectively induces cross-variant neutralisation (51). Our work now extends this by showing that strategies

328 to induce both S1 and S2 responses will also serve to boost ADCC. The polyspecific (*i.e.* S1 and S2) nature

329 of S-directed ADCC improves targeting of virus variants with heavily mutated S1 and may limit the ability

330 of viruses to evade this arm of host defense. With a wider range of Abs recognizing epitopes distributed

331 throughout S, the loss of any single epitope may not substantially impact ADCC, raising the barrier for

332 selection of escape mutants.

333 The S gene of seasonal human beta coronaviruses (HCoV HKU1, HCoV OC43) is divergent from SARS-CoV-

334 2, sharing only ~ 36% overall homology, with S2 somewhat more conserved than the S1 domain. The

335 preferential Ab targeting of S2 epitopes after infection may be partly attributed to a heterologous boost

336 towards cross-reactive S2-specific memory B cells (52-54), with pre-existing cross-reactive HCoV memory

337 B cells activated during SARS-CoV-2 infection kick-starting production of anti-S2 Abs. Interestingly, the

338 same regions we revealed as key regions of Ab reactivity corresponding with potent ADCC were previously

339 demonstrated to be elicited by heterologous anti-S Ab responses (54). This raises the question as to

340 whether immunodominant regions within S1 and S2 conserved between HCoV and SARS-CoV-2 are

341 selectively targeted for Ab reactivity and avidity to support robust ADCC. If SARS-CoV-2 infection induces

342 cross-reactive HCoV anti-S2 Ab production, it is unclear why vaccination did not have a similar effect.

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343 Prolonged antigen exposure from high viral loads, the presence of inflammatory stimuli during virus

344 infection, the way S traffics to and is presented on the infected cell surface, or differential sites of antigen

345 presentation (e.g. intramuscular for the vaccine, respiratory for the virus), may all contribute to diverse

346 outcomes from exposure through infection versus S subunit vaccination. It will be interesting whether

347 switching vaccine delivery from intra-muscular to intranasal, to promote mucosal immunity, impacts this

348 process (55, 56).

349 This study focussed on individuals who were infected prior to vaccination. Many individuals have now

350 been infected after being vaccinated with ancestral S antigen and it is unclear how these breakthrough

351 infections will affect ADCC against emerging variants. Our previous research demonstrated that infection

352 induces non-S ADCC responses that target N/M/ORF3a (11). However, as vaccination reduces the

353 induction of non-S (e.g. nucleocapsid) Ab during breakthrough infection, presumably due to enhanced

354 vaccine-mediated immunological control (57-59), breakthrough infections may fail to induce potent

355 N/M/ORF3a-mediated ADCC. In this case, strong S-directed ADCC induced by hybrid immunity would

356 become an important effector mechanism contributing to protection and an important consideration for

357 future research.

358 In summary, our data reveal that hybrid immunity establishes conditions whereby Abs generated against

359 key determinants within S1 and S2 domains elicit ADCC quantitatively superior to vaccination or infection

360 alone. In addition, hybrid immunity induced by ancestral antigen engenders an Ab response that retains

361 activity against variant strains to a far greater extent than neutralisation. Given the complementary roles

362 of neutralisation and ADCC in controlling cell-free and cell-associated virus, respectively, both Fc- and Fab

363 V-region mediated effector functions are desirable for incorporation into vaccine strategies. Strong ADCC

364 may play a part in the protection offered by hybrid immunity, uncovers a novel role for S2-targeted Abs,

365 and suggests that vaccine strategies based on spike expression would benefit from inducing Abs targeted

366 broadly across spike, as opposed to just RBD.

367 **Methods**

368 **Study subjects**

369 This study was carried out at two sites. In Canada, the study conformed to recommendations of the

370 Canadian Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans, and ethical

371 approval was given by the Health Research Ethics Authority of Newfoundland and Labrador (HREB).

372 Peripheral blood was collected from study subjects at approximately three-month intervals and a

373   questionnaire addressing previous testing history and reasons for suspecting infection with SARS-CoV-2

374   was administered at study intake after written informed consent in accordance with the Declaration of

375   Helsinki. Thirty-one individuals with confirmed infection who continued in the study and received 2 doses

376   of a COVID-19 vaccine were matched with 40 individuals with no previous infection history who had

377   received 2 doses of a COVID 19-vaccine (Table 1). Asymptomatic participants were identified through

378   Public Health surveillance and contact tracing after contact with RT-PCR-confirmed cases or through

379   serological testing for anti-S and anti-N protein IgG Abs (60). Most participants enrolled following the first

380   wave of COVID-19 and infections were attributed to ancestral Wuhan-Hu-1 SARS-CoV-2. Three

381   participants had infections attributed to B.1.1.7. Participants self-declared any medical treatments they

382   were receiving as well as information on comorbidity. Persons with any known underlying immune

383   compromising condition or on immunosuppressive treatment were excluded.

384   In the UK, ethical approval was given by Local Research Ethics Committee or the Avon Longitudinal Study

385   of Parents and Children (ALSPAC) Ethics and Law Committee. Peripheral blood was collected 2-4 weeks

386   after a RT-PCR or LFD confirmed infection, or after vaccination, from otherwise healthy donors.

387   Alternatively, serum samples submitted as part of the ALSPAC cohort (61-63) were assessed based on

388   serology for N and S, to identify individuals who had submitted samples after an initial infection, followed

389   by samples after each vaccination. In total 18 individuals who were infected prior to vaccination were

390   matched with 18 individuals who had received two doses of vaccines with no experience of prior infection.

391   Results were also compared to samples from a previously described cohort of individuals who had

392   experienced mild or severe COVID-19 and had not been vaccinated (11).

Blood sample processing

394   Whole blood was collected by venipuncture in acid citrate dextrose vacutainers, after which plasma was

395   collected following 10 min centrifugation at 500g and stored at -80°C (Canada). Alternatively, whole blood

396   was collected in a serum-separating vacutainer and serum collected following centrifugation at 500g for

397   10 min (UK). PBMC used for ADCC experiments were isolated from anti-coagulated heparinized blood

398   from healthy donors by density gradient centrifugation using the Canadian Autoimmunity Standardization

399   Core consensus standard operating procedure (version: March 21, 2019). Freshly isolated PBMC were

400   resuspended in lymphocyte medium consisting of RPMI-1640 with 10% FBS (HyClone™), 200 IU/mL

401   penicillin/streptomycin, 0.01 M HEPES, 1% L-glutamine (all from Invitrogen) and 2 x 10^{-5} M 2-

402   mercaptoethanol (Sigma-Aldrich) and used directly in functional experiments.

14

Cell lines and viruses

All cell lines and PBMC were cultured with 5% CO_2 at 37°C. VeroE6 cells expressing ACE2 and TMPRSS2 (VAT) and A549 cells expressing ACE2 (AA) were a gift from the University of Glasgow Centre for Virus Research (64). Human lung fibroblast MRC-5 cells were obtained from ATCC® (CCL-171™) and Lenti-X™ 293T cells were obtained from TakaRa (Mountain View CA, USA). All were propagated in complete DMEM (Sigma-Aldrich) containing 10% FBS (HyClone™) and 200 IU/mL penicillin/streptomycin (Invitrogen, Carlsbad CA, USA). Ancestral SARS-CoV-2 was recovered from a BAC containing the complete genome of a strain that matches the original Wuhan-Hu-1 (64) and the Omicron (BA.1) variant was a gift from Professor Arvind Patel (from the University of Glasgow Centre for Virus Research). Both were propagated in VAT cells. Virions were concentrated and purified by pelleting through a 30% sucrose cushion and titrated by plaque assay in AA cells as previously described (11). All virus seed stocks were verified by whole-genome sequencing on the Illumina platform.

Spike gene transfer and expression in MRC-5 cells

The recombinant Lenti-X™ pLVX-IRES lentiviral vector expression system (TakaRa) was used to introduce Wuhan-Hu-1, Delta (B.1.617.2), or Omicron (BA.1) S sequences into the MRC-5 cell line. The Wuhan-Hu-1 S was obtained from BEI Resources in a pcDNA™3.1(-) mammalian expression vector (NR-52420; NIAID, NIH) (65). Delta S was synthesised by Invitrogen GeneArt (Thermo Fisher Scientific) and contains mutations: T19R, T95I, G142D, E156G, E157- F158- R450L, K476T, G612D, R679P, N948D. Omicron S was obtained from BEI Resources in a pCMV/R mammalian expression vector (NR-56470; NIAID, NIH) with the following mutations: A67V, H69-, V70-, T95I, G142-, V143-, Y144-, Y145D, N211-, L212I, "EPE" insertion between 214R and 215D, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F.

Constructs were inserted into pLVX-IRES as detailed in (66) using conventional cloning protocols and all constructs were verified by forward and reverse strand sequencing (TCAG Facilities, Hospital for Sick Children, Toronto ON, Canada) to ensure authenticity. SnapGene Software (San Diego CA, USA) was used for designing and visualizing cloning procedures, designing and aligning sequencing primers and comparing variant sequences to wild-type Wuhan-Hu-1. Transduced cells were propagated and selected in complete DMEM containing 1 µg/mL puromycin (Sigma-Aldrich). Extracellular S expression was confirmed by flow cytometry, as previously outlined (66).

15

Antibody-dependent cell-mediated killing assays

PBMC were freshly processed, resuspended in lymphocyte medium and kept at 37°C and 5% CO_2 until use. For ADCC assays using Wuhan-Hu-1, Delta or Omicron S-expressing MRC-5 target cells, 10^4 cells/well were plated and labelled with 1 µCi $Na_2{}^{51}CrO_4$/well (PerkinElmer, Akron, OH, USA) overnight in 96-well round bottom plates and then washed four times in PBS containing 1% FBS (HyClone™). PBMC (E:T 25:1) and heat-inactivated plasma (56°C for 1h) were added to wells with a final volume of 300 µL and final plasma dilution of 1:1000 and cytotoxic activity measured by ^{51}Cr release over 5 h. ^{51}Cr release was measured in 125 µL of supernatant on a Wallac 1480 Wizard gamma counter and percent specific lysis calculated by (experimental ^{51}Cr release − spontaneous ^{51}Cr release) / (maximum ^{51}Cr release − spontaneous ^{51}Cr release) x 100.

Assays using virus-infected cells used CD107a degranulation ADNKA as a proxy for ADCC to be in compliance with BLS3 containment and were carried out as previously described (11). Briefly, AA cells were infected at MOI = 5 for 24 h prior to being detached with TrypLE™ (Thermo Fisher, Waltham, MA USA), then 25,000 targets mixed with 250,000 PBMC, serum, and anti-CD107a-FITC (BioLegend, London, UK) and GolgiStop (BD, Swindon, UK) in a total volume of 100 µL for 5 h. PBMC were stained with live/dead fixable aqua (Thermo Fisher), anti-CD3-PE-Cy7 (UCHT1, BioLegend), anti-CD56-BV605 (5.1H11, BioLegend), and anti-CD57-APC (HNK-1, BioLegend). Data were acquired and analysed using an Attune NXT Flow Cytometer (Thermo Fisher) and expressed as the percentage of live CD107a$^+$ CD57$^+$ NK (CD3$^-$ CD56$^+$) cells. All sera were tested against mock-infected cells to ensure there was no background NK cell activation and any sera demonstrating background activation were excluded. A seronegative serum was included in all assays as a negative control. To enable comparisons with previous datasets, and to minimise inter-experiment variability, a donor serum demonstrating moderate ADNKA was included as a positive control in every assay. Sera were tested at a range of dilutions, and the area under the curve (AUC) was calculated using GraphPad Prism 9. This value was then normalised to the AUC for the standard serum in each assay.

Virus neutralisation assay

Assays were carried out as previously described (11). Briefly, 600 plaque forming units of SARS-CoV-2 was incubated with appropriate dilutions of serum, in duplicate, for 1 h at 37°C. The mixes were then added to pre-plated VeroE6 cells for 48 h. After this time, monolayers were fixed with 4% paraformaldehyde (Thermo Fisher), permeabilised for 15 min with 0.5% NP-40 (Merck Life Science, Dorset, UK) then blocked for 1 h in PBS containing 0.1% Tween (PBST) and 3% non-fat milk. Primary Ab (anti-N 1C7, Stratech, 1:500

16

464   dilution) was added in PBST containing 1% non-fat milk and incubated for 1 h at room temperature. After

465   washing in PBST, secondary Ab (anti-mouse IgG-HRP, Pierce, 1:3000 dilution) was added in PBST

466   containing 1% non-fat milk and incubated for 1 h. Monolayers were washed again, developed using

467   Sigmafast™ OPD (Sigma-Aldrich) according to manufacturers' instructions, and read on a Clariostar Omega

468   plate reader (OD 450 nm). Wells containing no virus, virus but no Ab, and a standardized serum displaying

469   moderate activity were included as controls in every experiment. NT50 were calculated in GraphPad Prism

470   9.

Serological testing

472   Plasma was diluted in PBS containing 0.05% TWEEN® 20 (0.05% PBST; Sigma-Aldrich, St. Louis, MO, USA)

473   and 0.1% bovine serum albumin (BSA, Sigma-Aldrich) then tested against recombinant proteins coated in

474   Dulbecco's PBS (DPBS, Sigma-Aldrich) overnight onto 96-well Immunlon-2 plates (VWR Scientific,

475   Mississauga, ON, Canada). Recombinant protein antigens included SARS-CoV-2 FLS glycoprotein trimer

476   [50 ng/well; SMT1-1 reference material, National Research Council (NRC), Canada], the S1 subunit of

477   SARS-CoV-2 S (65 ng/well; SinoBiological, Wayne, PA, USA) and S2 subunit of SARS-CoV-2 S (50 ng/well;

478   SinoBiological). The predicted molecular mass for S1 and S2 were 76.5 kDa and 59.4 kDa, respectively, and

479   coating amounts were determined to account for this difference. Plates were washed 4 times with 0.05%

480   PBST, blocked for 1 h with 200 µL of PBS containing 1% BSA (Sigma-Aldrich), washed 4 times then 100

481   µL/well of diluted plasma (1:500 for FLS IgG or 1:100 for S1 and S2 IgG and IgG$_3$) was applied to antigen-

482   coated plates in duplicate wells for 1.5 h. Total IgG was measured following 6 washes and a 1-h incubation

483   with 100 µL/well of 1:50,000 HRP-conjugated polyclonal goat anti-human IgG (Jackson ImmunoResearch

484   Labs, West Grove, PA, USA). IgG$_3$ was measured following 6 washes and a 1-h incubation with 100 µL/well

485   1:5,000 mouse anti-human biotin-conjugated IgG$_3$ hinge (SouthernBiotech, Birmingham, Alabama, USA)

486   then 6 washes and 1-h incubation with 100 µL/well 1:40,000 HRP-conjugated streptavidin (Jackson

487   ImmunoResearch Labs). Plates were developed using tetramethylbenzidine (TMB) substrate (Sigma

488   Aldrich) following 6 washes then incubated in the dark at room temperature for 20 min. Reactions were

489   stopped with an equal volume of 1 M H$_2$SO$_4$ and optical density (OD) read on a BioTek synergy HT plate

490   reader at 450 nm.

Antibody depletions

492   Depletions of specific Abs from sera were carried out as previously described (11). In brief, Abs targeting

493   different domains of S were depleted using magnetic bead conjugated proteins based on either the RBD,

494   NTD, entire S1, or entire S2 domain of SARS-CoV-2 Wuhan-Hu-1 S (ACROBiosystems). Beads were

17

495   resuspended in PBS + 0.05% BSA, then serum was pre-diluted 1:9, and 50 µl mixed with 150 µl beads.

496   Mixtures were incubated on a rotating mixer at 4 °C overnight. Serum diluted in buffer alone was used as

497   a control. Magnetic beads were then removed using a 3D printed magnetic stand then a second round of

498   depletion carried out using fresh beads as indicated. All values in assays were corrected for dilutions.

499   **Peptide scan ELISA**

500   Individual overlapping peptides (17- or 13-mers, with 10 amino acid overlaps) spanning the canonical

501   Wuhan-Hu-1 S sequence (NR-52402; BEI Resources) were reconstituted at 10 mg/mL in DMSO (Sigma-

502   Aldrich) then diluted to 50 µg/mL in DPBS (Sigma-Aldrich) and stored at -20°C. 125 ng/well of each S

503   peptide 2 – 181 (BEI Resources) was coated overnight on Immunlon-2 plates (VWR Scientific) in sequential

504   pairs (e.g. 2 & 3… 180 & 181). Full length S trimer (SMT1-1, NRC) was diluted in DPBS and coated overnight

505   at 150 ng/well as positive control. Plates were washed four times with 0.05% PBST and blocked for 1 h

506   with 200 µL/well PBS + 1% BSA. Plasma was diluted 1:50 in 0.05% PBST + 0.1% BSA and 50 µL was applied

507   for 1.5 h. Plates were washed six times and total IgG binding was measured in a 1-h incubation with 100

508   µL/well of 1:50,000 HRP-conjugated polyclonal goat anti-human IgG (Jackson ImmunoResearch Labs) and

509   developed using 50 µL/well TMB substrate (Sigma Aldrich). Reactions were stopped with an equal volume

510   of 1 M H_2SO_4 and OD read on a BioTek synergy HT plate reader at 450 nm.

511   **Statistical analysis**

512   Statistical analyses were performed using GraphPad Prism 9 with two-sided *P*-values < 0.05 considered

513   significant. Normality of data distributions were assessed using Shapiro-Wilk test. Significance in

514   correlations were assessed using Spearman's rank correlation coefficient. Differences in means with

515   standard deviation (SD) or medians with interquartile range (IQR, calculated as IQR = $Q_3 - Q_1$) between

516   groups were compared by using one-way ANOVA, Student's *t*-test, Friedman test or Mann-Whitney *U*-test

517   as appropriate based on normality of data distribution.

Competing Interests

519   The authors declare no competing interests.

Author Contributions

521   KAH, RJS, CAF and KB designed and conducted the experiments, analyzed data, constructed figures and

522   illustrations and wrote/edited the manuscript. MDG and EW co-wrote and edited the manuscript. KMH

523   and DPI assisted with experiments. DH coordinated patient consent, questionnaires and collected blood

524   samples. MDG, EW, RJS and KAH obtained funding.

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Data Availability Statement

540   Data supporting the findings of this study and preserving anonymity of study participants will be made

541   available from the corresponding authors through electronic correspondence for legitimate scientific

542   purposes.

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23

687   **Figure Captions**

688   **Figure 1| Vaccine-, infection-, and hybrid immunity-elicited S-specific NK cell activation.**

689   Background NK cell lysis of (a) non-transduced (open black circle) or Wuhan-Hu-1 S-expressing (red

690   square) MRC-5 cells elicited by plasma from SARS-CoV-2 seropositive (n = 10) or plasma collected pre

691   pandemic (n = 8), respectively, was measured by ^{51}Cr release (E:T 25:1). (b) Sequential measures of S-

692   MRC-5 cell ADCC elicited by plasma collected after infection then subsequent vaccination or after

693   vaccination alone was measured by ^{51}Cr release (E:T 25:1). Experiments were performed in duplicate with

694   three independent donors and a representative plot shown. (c, d) Serum samples were serially diluted

695   and CD57^{+} NK cell CD107a degranulation against A549-ACE2 cells infected with SARS-CoV-2 24 h

696   previously at MOI = 5 was measured by flow cytometry. Data from a single individual is shown in (c) and

697   compiled data from multiple donors in (d) was assessed by calculating the area under the curve (AUC).

698   Black lines bisecting groups in (a) represent the mean of individual plasma samples with standard

699   deviation, and in (b) represent median with IQR. *P* value in (b) was calculated using Kruskal-Wallis test

700   with Dunn's multiple comparisons test and in (d) calculated using one-way ANOVA for matched data with

701   Tukey's correction and shown above horizontal lines spanning comparison groups when significant. The

702   probability of hybrid immunity inducing more robust ADCC than vaccine-induced immunity was calculated

703   using two-sided Fisher's exact test.

704   **Figure 2| Spike-directed ADNKA against SARS-CoV-2-infected cells.** A549-ACE2 cells were infected

705   with SARS-CoV-2 for 24 h and CD57^{+} NK cell CD107a expression measured in response to serial dilutions

706   of sera from vaccinees (PV2), hybrid immunity (PV2) or persons recovered from mild or severe infection

707   and no vaccination. Representative data in (a) is depicted at the indicated dilutions and (b) the AUC was

708   calculated, and data compiled for multiple donors (vaccinee n = 18; hybrid immunity n = 18; mild infection

709   n = 18; severe infection n = 14). (c) Sera used in (b) were applied to SARS-CoV-2-infected cells,

710   neutralisation assessed and NT50 calculated. (d, e) The significance of correlations between ADNKA and

711   NT50 were assessed using Spearman's correlation.

712   **Figure 3| Associations between ADCC and anti-S1/S2 IgG and IgG$_3$ Ab abundance.** Correlations

713   between ADCC induced by plasma Ab from samples collected post infection (PI) and levels of anti-S1 (open

714   black circle) and anti-S2 (red square) (a) IgG and (b) IgG$_3$ are plotted. Relationships between the magnitude

715   of ADCC mediated by hybrid (PV1; open black circle) and vaccinee (PV2; black square) plasma Ab and (c)

716   anti-FLS IgG, (d) anti-S1 IgG, (e) anti-S2 IgG or (f) anti-FLS IgG$_3$, (g) anti-S1 IgG$_3$ and (h) anti-S2 IgG$_3$ are

717   depicted.

24

718 **Figure 4| Anti-S1/S2 Ab responses after vaccination, infection, and hybrid immunity.** Circulating

719 anti-FLS IgG Abs from (a) participants with hybrid immunity were measured post infection (PI; blue circle)

720 and after first (PV1; red chevron) and second (PV2; green square) vaccinations, and from vaccinated

721 participants (n = 40) PV1 (purple triangle) and PV2 (orange diamond). Levels of anti-S1 IgG (open black

722 circle) and anti-S2 IgG (red square) in samples obtained from (b) participants with hybrid immunity were

723 compared PI, PV1 and PV2, and from (c) vaccinees were compared PV1 and PV2. Relative amounts of anti-

724 S1 and anti-S2 IgG_3 were measured for (d) persons with hybrid immunity or (e) vaccinees and anti-S1 and

725 anti-S2 IgG_3 Ab levels compared. Hybrid- and vaccine-induced anti-S1 and anti-S2 (f) IgG and (g) IgG_3 were

726 contrasted. P values in (a) were calculated using Kruskal-Wallis test with Dunn's multiple comparisons

727 test; (b) – (e) using Friedman's test with Dunn's multiple comparisons test; (f), (g) using Kruskal-Wallis test

728 with Dunn's multiple comparisons test and are shown above horizontal lines spanning comparison groups

729 when significant. Lines bisecting groups represent median with IQR.

730 **Figure 5| Assessment of ADNKA after S subunit Ab depletion.** Sera from individuals with hybrid

731 immunity were diluted 1:9 then Abs targeting the indicated domains of S were depleted using magnetic

732 bead-conjugated protein. These depleted serums were then used to measure $CD57^+$ NK cell CD107a

733 expression at the indicated dilutions in the presence of SARS-CoV-2-infected A549-ACE2. Four individuals

734 are depicted in (a) – (d). (e) The AUC for each sample in (a) – (d) was calculated then the amount of ADNKA

735 remaining following depletion of Abs targeting each domain in relation to sera containing all Abs, or sera

736 from which all S Abs had been depleted, was determined (n = 5).

737 **Figure 6| Peptide scanning to identify distinct linear regions associated with robust ADCC.**

738 Linear FLS Ab epitope reactivity was determined by ELISA-based peptide scanning. (a) A representative

739 depiction of anti-IgG Ab reactive to linear segments is illustrated by an overlaid line graph. The blue line

740 represents OD results of the full S peptide scan of a sample collected from one participant recovered from

741 moderate COVID-19 infection and the orange overlay represents a sample from the same participant

742 collected 1 month after their first vaccination. (b) Compiled peptide scan data from samples collected

743 from 7 participants post infection (PI) and after hybrid immunity (H) were illustrated using a heat map and

744 aligned with known mutations in Delta and Omicron sequences. Three determinants associated with ADCC

745 are identified by asterisks. (c) The left axis depicts anti-S1 CDT1/2 (triangle), S1 CTD2 (circle) and S2 CD

746 (square) IgG Ab OD and compares these levels with ADCC (right axis) for two participants after infection

747 (open black bar) and after subsequent vaccination (open red bar). Participant IgG Ab OD collected from

25

748   peptide scanning were (d) scored for number of distinct regions or (e) tallied and associations between

749   Ab reactivity and levels of ADCC assessed.

750   **Figure 7| Vaccine- and hybrid-induced S-specific ADCC against variants of concern.** (a) Histogram

751   overlay of MRC-5 cells transduced to express similar levels of Wuhan-Hu-1 (black), Delta (grey) and

752   Omicron (red) S protein used in ^{51}Cr assay to assess the efficacy of Ab produced with (b) hybrid (n = 14) or

753   (c) vaccine-induced (n = 16) immunity in eliciting ADCC against variant strains. (d) SARS-CoV-2

754   neutralisation assay was performed on serial dilutions of serum samples and NT50 values against either

755   Wuhan-Hu-1 or Omicron-infected A549-ACE2 were calculated. (e) Serum samples were serially diluted

756   and CD57$^+$ NK cell CD107a expression against A549-ACE2 cells infected with either Wuhan-Hu-1 or

757   Omicron was measured by flow cytometry and AUC calculated. P values in (b) - (e) were calculated using

758   Student's paired *t* test and shown above horizontal lines spanning comparison groups when significant.

Tables

Table 1| Relevant features and categorization of study cohort

		Hybrid n = 31	Vaccinee n = 40
Symptoms n (%)	Asymptomatic	4 (12.9)	N/A
	Mild	8 (25.8)	
	Moderate	14 (45.2)	
	Severe	5 (16.1)	
	Mean DPI/DPSI ± SD*	250.4 ± 103.2	
	Median Age (IQR)	63 (53-73)	55 (40-66)
	Mean Age ± SD	60 ± 15.2	53.8 ± 14.3
	Female n (%)	18 (58)	27 (67.5)
	Male n (%)	13 (42)	13 (32.5)
Vaccine 1	ChAdOx1-S n (%)	3 (9.7)	4 (10)
	Pfizer-BioNTech n (%)	26 (83.9)	34 (85)
	Moderna n (%)	2 (6.4)	2 (5)
	Mean DPV1 ± SD*	58 ± 14.8	61 ± 15.4
Vaccine 2	Pfizer-BioNTech n (%)	20 (64.5)	26 (65)
	Moderna n (%)	11 (35.5)	14 (35)
	Mean DPV2 ± SD*	71.2 ± 21.1	72 ± 17.7
	Mean Days Between Vaccination ± SD	71.3 ± 16.8	75.5 ± 16

*Days post infection/days post symptom onset (DPI/DPSO); days post first vaccination (DPV1); days post second vaccination (DPV2). Mild = few symptoms < 7 days; moderate = multiple symptoms > 7 days; severe = hospitalised.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7