1   # The BNT162b2 mRNA vaccine against SARS-CoV-2 reprograms both adaptive and

2   # innate immune responses

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36

Summary

38

39   The mRNA-based BNT162b2 vaccine from Pfizer/BioNTech was the first registered COVID-19

40   vaccine and has been shown to be up to 95% effective in preventing SARS-CoV-2 infections.

41   Little is known about the broad effects of the new class of mRNA vaccines, especially whether

42   they have combined effects on innate and adaptive immune responses. Here we confirmed

43   that BNT162b2 vaccination of healthy individuals induced effective humoral and cellular

44   immunity against several SARS-CoV-2 variants. Interestingly, however, the BNT162b2 vaccine

45   also modulated the production of inflammatory cytokines by innate immune cells upon

46   stimulation with both specific (SARS-CoV-2) and non-specific (viral, fungal and bacterial) stimuli.

47   The response of innate immune cells to TLR4 and TLR7/8 ligands was lower after BNT162b2

48   vaccination, while fungi-induced cytokine responses were stronger. In conclusion, the mRNA

49   BNT162b2 vaccine induces complex functional reprogramming of innate immune responses,

50   which should be considered in the development and use of this new class of vaccines.

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53   **Keywords**: COVID-19, coronaviruses, mRNA vaccines, trained immunity, innate immune

54   tolerance

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Main text

Coronavirus disease 2019 (COVID-19) is a new respiratory tract infection caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which spread worldwide since the end of 2019 causing a global pandemic. The COVID-19 pandemic represents the most important healthcare crisis humanity has encountered since World War II, combined with a devastating societal and economic impact. Confronted with this critical situation, a major effort to develop vaccines against COVID-19 has been initiated in many countries around the world.

To date, 13 vaccines have been approved for use in humans ("COVID-19 vaccine tracker | RAPS," n.d.). The scale of the pandemic has led to accelerated development of vaccines based on new technologies, such as mRNA- and viral vector-based vaccines (van Riel & de Wit, 2020). One of the most widely used anti-COVID-19 vaccines in the world was developed by a collaboration between BioNTech and Pfizer (BNT162b2). This vaccine is based on a lipid nanoparticle–formulated, nucleoside-modified mRNA that encodes a prefusion stabilized form of the spike (S)-protein derived from the SARS-CoV-2 strain isolated early on in Wuhan, China (Walsh et al., 2020). Several phase-3 trials have demonstrated that BNT162b2 elicits broad humoral and cellular responses, providing protection against COVID-19 (Sahin et al., 2020; Walsh et al., 2020).

While global vaccination campaigns against the SARS-CoV-2 infection are rolled out, major challenges remain, especially the spread of novel virus variants (Madhi et al., 2021). One of the most prominent mutations during the pandemic has been the spike D614G substitution to the Wuhan Hu-1 original strain (Korber et al., 2020). With the steadily increasing prevalence of

3

80   infections, SARS-CoV-2 variants emerged with multiple spike mutations and were first detected

81   in the United Kingdom (B.1.1.7 lineage), South Africa (B.1.351 lineage), and Brazil (P.1 lineage).

82   These variants are of significant concern because of their potential effects on disease severity,

83   viral transmissibility, reinfection rates, and vaccine effectiveness (Abdool Karim & de Oliveira,

84   2021).

85

86   The capacity of BNT162b2 to induce effective humoral and cellular immunity against the new

87   SARS-CoV-2 variants is only now beginning to be understood. Whereas neutralization of B.1.1.7

88   was either similar or just slightly reduced as compared to the standard strain (Muik et al., 2021;

89   Wang, Nair, et al., 2021), neutralizing titers of B.1.351 were markedly diminished (Liu et al.,

90   2021; Planas et al., 2021; Wang, Nair, et al., 2021) after vaccination of healthy volunteers with

91   BNT162b2. In contrast, cellular immunity against the virus variants seems to be less affected

92   (Lilleri et al., 2021; Skelly et al., 2021). In addition, an unexplored area is whether vaccination

93   with BNT162b2 also leads to long-term effects on innate immune responses: this could be very

94   relevant in COVID-19, in which dysregulated inflammation plays an important role in the

95   pathogenesis and severity of the disease (Tahaghoghi-Hajghorbani et al., 2020). The long-term

96   modulation of innate immune responses has been an area of increased interest in the last

97   years: multiple studies have shown that long-term innate immune responses can be either

98   increased (*trained immunity*) or down-regulated (*innate immune tolerance*) after certain

99   vaccines or infections (Netea et al., 2020).

100

101   In this study, we assessed the effect of the BNT162b2 mRNA COVID-19 vaccine on both the

102   innate and adaptive (humoral and cellular) immune responses. We first examined the

103   concentration of RBD- and S-binding antibody isotype concentrations before vaccination

104   (baseline; t1), 3 weeks after the first dose of 30 µg of BNT162b2 (t2), and two weeks after the

105   second dose (t3) (Figure S1A). We calculated fold-changes by comparing concentrations from

106   both t2 and t3 to baseline. BNT162b2 vaccination elicited high anti-S protein and anti-RBD

107   antibody concentrations already after the first vaccination, and even stronger responses after

108   the second dose of the vaccine. As expected, IgG responses were the most pronounced, with

109   RBD-specific median fold changes at t2 and t3 of 56-fold and 1839-fold, and S-specific fold

110   changes of 208-fold and 1100-fold, respectively. The lowest observed fold change increase to

111   pre-vaccination levels of IgG targeting RBD was 14-fold at t2, and 21-fold at t3, respectively. For

112   S-specific IgG, the fold changes were at least 32-fold at t2 and 339-fold at t3. Regarding IgA

113   concentrations, a single dose of the vaccine elicited a 7-fold increase in the RBD-specific

114   concentration and a 35-fold increase in the S-specific concentration. The second dose

115   enhanced the antibody concentrations elicited by the first vaccination by 24-fold and 52-fold,

116   for RBD and S, respectively. Compared to IgG and IgA, the increase in IgM concentrations was

117   considerably lower. RBD-specific concentration only doubled after the first dose, and it did not

118   further increase after the second dose of the vaccine. In contrast, S-specific fold changes were

119   11-fold at t2 and 20-fold at t3 (Figure S1A). These results confirm and extend recent

120   observations reporting strong induction of humoral responses by BNT162b2 vaccination (Sahin

121   et al., 2020).

122

123   To investigate the neutralizing capacity of the serum against SARS-CoV-2 variants, we

124   performed 50% plaque reduction neutralization testing (PRNT50) using sera collected two

125   weeks after the second vaccine administration. All the serum samples neutralized the D614G

126   strain and the B.1.1.7 variant with titers of at least 1:80. However, six subjects (37,5%) had titers

127   lower than 1:80 against the B.1.351 variant. Geometric mean neutralizing titers against the

128   D614G strain, B.1.1.7 and B.1.351 were 381, 397, and 70, respectively (Figure S1B, p<0.001).

129   Similar to our investigation, several studies reported 6 to 14-fold decreased neutralizing activity

130   of post-vaccine sera against the B.1.351 variant, and only slightly reduced activity against

131   B.1.1.7, when compared to the standard strain (Planas et al., 2021; Shen et al., 2021; Wang,

132   Liu, et al., 2021). These data support the evidence that B.1.351, and possibly other variants,

133   may be able to escape vaccine-induced humoral immunity to a certain extent (Kustin et al.,

134   2021). Furthermore, the PRNT titer and the antibody concentrations of IgG after the second

135   dose were strongly correlated (Figure S1C). The correlation was stronger for B.1.1.7 and B.1.351

136   than for the standard strain, both for anti-RBD and anti-S.

137

138   BNT162b2 vaccination has been reported to activate virus-specific CD4+ and CD8+ T cells, and

139   upregulate the production of immune-modulatory cytokines such as IFN-γ (Sahin et al., 2020).

140   Hence, we assessed IFN-γ secretion from peripheral blood mononuclear cells (PBMCs) before

141   and after BNT162b2 vaccination in response to heat-inactivated SARS-CoV-2 strains (Figs 2A-

142   2D). While vaccination with BNT162b2 generally seems to moderately increase specific IFN-γ

143   production after the second dose of the vaccine, this reached statistical significance only upon

                                   S. African

144   stimulation with B.1.351 variant (Figure S2A-2C). The same tendency has also been observed

145   by Tarke et al. who used synthetic SARS-CoV-2 variants proteins to induce elevated IFN-γ

146   responses against B.1.351 (Tarke et al., 2021). IFN-γ production was higher by at least 50% in

147   37.5% of the subjects upon stimulation with the standard SARS-CoV-2 strain, in 50% of the

148   subjects upon stimulation with the B.1.1.7 variant and the B.1.351 variant, but only in 18,75%

149   of the subjects upon stimulation with the Bavarian variant (Figure S1D). These findings argue

150   that BNT162b2 vaccination induces better humoral than cellular immune responses. Weak T-

151   cell responses have previously been reported in vaccinees that have received just a single dose

152   of BNT162b2 (Prendecki et al., 2021; Stankov, Cossmann, Bonifacius, Dopfer-jablonka, &

153   Morillas, 2021). Intriguingly, the best cellular responses after vaccination were against the

154   B.1.351 variant: the fact that the neutralizing antibody responses against this variant were

155   relatively poor, that may raise the possibility that the protective BNT162b2 vaccine effects

156   against this variant may be mainly reliant on cellular, rather than humoral, responses. No

157   significant differences between the individual variants were observed. The absolute

158   concentrations of the cytokines after stimulations can be found in Supplementary Table 2.

159

160   Interestingly, we observed important heterologous effects of BNT162b2 vaccination on IFN-γ

161   production induced by other stimuli as well (Figures S2E, 2F). BNT162b2 vaccination decreased

162   IFN-γ production upon stimulation with the TLR7/8 agonist R848 (Figure S2F). In contrast, the

163   IFN-γ production induced by inactivated influenza virus tended to be higher two weeks after

164   the second BNT162b2 vaccination, though the differences did not reach statistical significance.

165   We did not find any significant correlation between cellular responses and IgG antibody titers.

166

167   Besides their effects on specific (adaptive) immune memory, certain vaccines such as Bacillus

168   Calmette-Guérin (BCG) and the measles, mumps, and rubella (MMR) vaccine also induce long-

169   term functional reprogramming of cells of the innate immune system. (Netea et al., 2020). This

170   biological process is also termed *trained immunity* when it involves increased responsiveness,

171   or *innate immune tolerance* when it is characterized by decreased cytokine production (Ifrim

172   et al., 2014). Although these effects have been proven mainly for live attenuated vaccines, we

173   sought to investigate whether the BNT162b2 vaccine might also induce effects on innate

174   immune responses against different viral, bacterial and fungal stimuli. One of the trademarks

175   of trained immunity is an elevated production of inflammatory cytokines following a secondary

176   insult (Quintin et al., 2012). Surprisingly, the production of the monocyte-derived cytokines

177   TNF-α, IL-1β and IL-1Ra tended to be lower after stimulation of PBMCs from vaccinated

178   individuals with either the standard SARS-CoV-2 strain or heterologous Toll-like receptor

179   ligands (Figures 1 and 2). TNF-α production (Figure 1B-1G) following stimulation with the

180   TLR7/8 agonist R848 of peripheral blood mononuclear cells from volunteers was significantly

181   decreased after the second vaccination (Figure 1C). The same trend was observed after

182   stimulation with the TLR3 agonist poly I:C (Figure 1D), although the difference did not reach

183   statistical significance. In contrast, the responses to the fungal pathogen *Candida albicans* were

184   higher after the first dose of the vaccine (Figure 1G). The impact of the vaccination on IL-1β

185   production was more limited (Figure 2A-2F), though the response to *C. albicans* was

186   significantly increased (Figure 2F). The production of the anti-inflammatory cytokine IL-1Ra

187   (Figure 2G-2L) was reduced in response to bacterial lipopolysaccharide (LPS) and *C. albicans*

188   after the second vaccination (Figure 2K, 2L), which is another argument for a shift towards

189   stronger inflammatory responses to fungal stimuli after vaccination. IL-6 responses were

190   similarly decreased, though less pronounced (data not shown).

191

192   The induction of tolerance towards stimulation with TLR7/8 (R848) or TLR4 (LPS) ligands by

193   BNT162b2 vaccination may indicate a more balanced inflammatory reaction during infection

194   with SARS-CoV-2, and one could speculate whether such effect may be thus useful to regulate

195   the potential over-inflammation in COVID-19, one of the main causes of death (Tang et al.,

196   2020). On the other hand, inhibition of innate immune responses may diminish anti-viral

197   responses. Type I interferons also play a central role in the pathogenesis and response against

198   viral infections, including COVID-19 (Hadjadj et al., 2020). With this in mind, we also assessed

199   the production of IFN-α by immune cells of the volunteers after vaccination. Although the

200   concentrations of IFN-α were below the detection limit of the assay for most of the stimuli, we

201   observed a significant reduction in the production if IFN-α secreted after stimulation with poly

202   I:C and R848 after the administration of the second dose of the vaccine (Figure 1H, 1I). This

203   may hamper the initial innate immune response against the virus, as defects in TLR7 have been

204   shown to result in and increased susceptibility to COVID-19 in young males (Van Der Made et

205   al., 2020). These results collectively demonstrate that the effects of the BNT162b2 vaccine go

206   beyond the adaptive immune system and can also modulate innate immune responses.

207

208   The effect of the BNT162b2 vaccination on innate immune responses may also indicate a

209   potential to interfere with the responses to other vaccinations, as known for other vaccines to

210   be as 'vaccine interference' (Lum et al., 2010; Nolan et al., 2008; Vajo, Tamas, Sinka, &

211   Jankovics, 2010). Future studies are therefore needed to investigate this possibility, especially

212   the potential interaction with the influenza vaccine: in the coming years (including the autumn

213   of 2021) COVID-19 vaccination programs will probably overlap with the seasonal Influenza

214   vaccination, so it is crucial to perform additional studies to elucidate the potential interactions

215   and effects of the COVID-19 vaccines with the current vaccination schedules, especially for

216   immunosuppressed and elderly individuals.

217

218   The generalizability of these results is subject to certain limitations. First, the number of

219   volunteers in this study was relatively small, although in line with earlier immunological studies

220   on the effects of COVID-19 vaccines. Second, our cohort consisted of healthcare workers, who

221   are middle-aged and healthy, and future studies in elderly individuals and people with

222   comorbidities and other underlying risk factors for severe COVID-19 infections need to be

223   performed (Gao et al., 2021). Third, our study is performed only with individuals with a Western

224   European ancestry. Therefore, the conclusions of our study should be tested in populations

225   with different ancestry and alternative lifestyles since the induction of innate and adaptive

226   immune responses is largely dependent on different factors such as genetic background, diet,

227   and exposure to environmental stimuli which largely differ between communities around the

228   globe.

229

230   In conclusion, our data show that the BNT162b2 vaccine induces effects on both the adaptive

231   and the innate branch of immunity and that these effects are different for various SARS-CoV-2

232   strains. Intriguingly, the BNT162b2 vaccine induces reprogramming of innate immune

233   responses as well, and this needs to be taken into account: in combination with strong adaptive

234   immune responses, this could contribute to a more balanced inflammatory reaction during

235   COVID-19 infection, or it may contribute to a diminished innate immune response towards the

236   virus. BNT162b2 vaccine is clearly protective against COVID-19, but the duration of this

237   protection is not yet known, and one could envisage future generations of the vaccine

238   incorporating this knowledge to improve the range and duration of the protection. Our findings

239   need to be confirmed by conducting larger cohort-studies with populations with diverse

240   backgrounds, while further studies should examine the potential interactions between

241   BNT162b2 and other vaccines.

242

243   ## Acknowledgements

244

253   ## Author contributions

254   Conceptualization: M.G.N, F.K.F, J.t.O, J.H., R.v.C, J.v.d.M., F.v.d.V. and L.A.B.J.; Clinical

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256   H.D., H.L., S.A.S, M.R.; Supervision: R.P.v.R., M.I.d.J., J.D-A. and M.G.N. Writing and correction
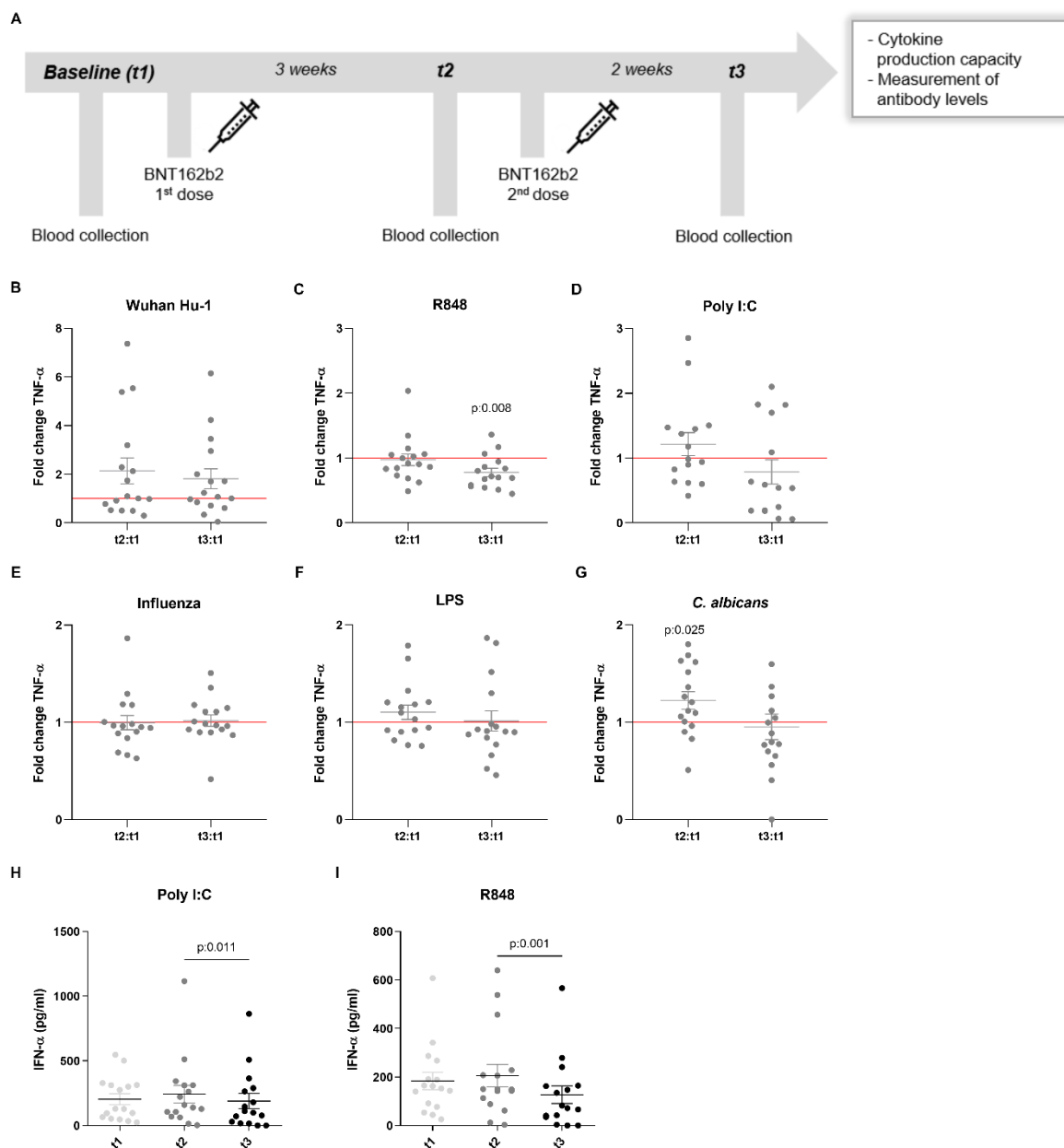
257   of the manuscript: all authors.

258

259   ## Declaration of Interests

260   M.G.N and L.A.B.J are scientific founders of Trained Theraputix and Discovery.

261

262

263   # Main figures

264

265   Figure 1. TNF-α and IFN-α production in response to heterologous stimuli in PBMCs isolated

266   from vaccinated subjects. (A) Description of the study: vaccination and blood collection days.

267   (B-G) Fold change values of TNF-α production are calculated individually for each subject by

268   division of t2:t1 and t3:t1. Data are presented as fold changes ± SEM (n=15-16) and analysed

269   by Wilcoxon's matched-pairs signed-rank test comparing each ratio to t1=1 (red line). (H-I)

270   IFN-α production (pg/ml) at t1, t2 and t3. Data are presented as cytokine concentration ± SEM

271   (n=15-16) and analysed by Wilcoxon's matched-pairs signed-rank test.

12
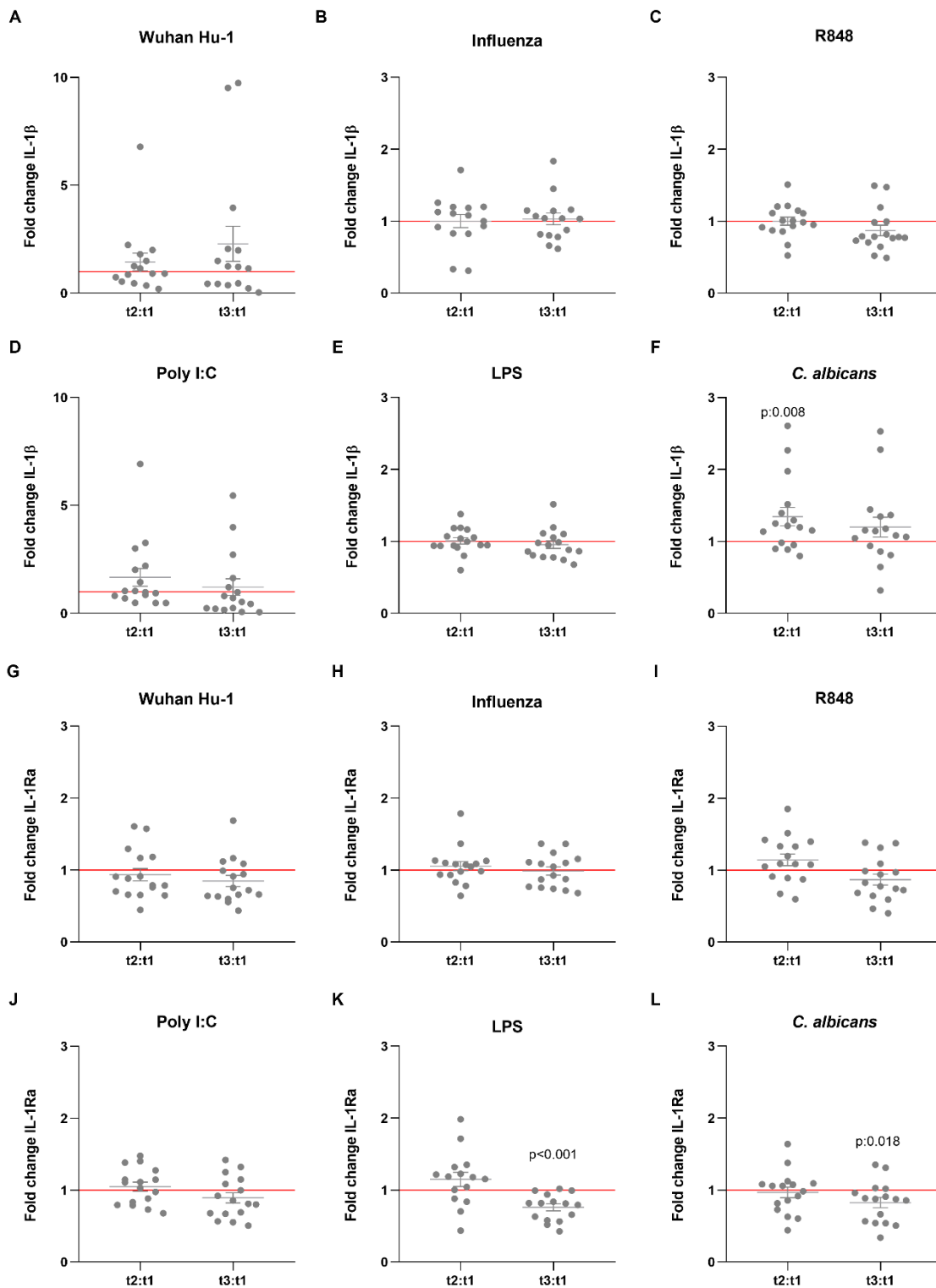
Figure 2. IL-1β and IL-1Ra production in response to heterologous stimuli in PBMCs isolated from vaccinated subjects. IL-1β (A-F) and IL-1Ra (G-L) Fold change values are calculated individually for each subject by division of t2:t1 and t3:t1. Data are presented as fold changes ± SEM (n=15-16) and analysed by Wilcoxon's matched-pairs signed-rank test comparing each ratio to t1=1 (red line).

13

278   ## Methods

279

280   ### Resource availability

281   ### Lead contact

282   Further information and requests for resources and reagents should be directed to and will be

283   fulfilled by the Lead Contact Mihai G. Netea (mihai.netea@radboudumc.nl).

284

285   ### Materials availability

286   This study did not generate new unique reagents.

287

288   ### Data and code availability

289   Data from this study are available upon request.

290

291   ### Experimental model and subject details

292   ### Human subject collection

293   The study was conducted in compliance with ethical principles of the Declaration of Helsinki,

294   approved by the Arnhem-Nijmegen Institutional Review Board (protocol NL76421.091.21) and

295   registered in the European Clinical Trials Database (2021-000182-33). Health care workers from

296   the Radboudumc Nijmegen were enrolled who received the BNT162b2 mRNA Covid-19 vaccine

297   as per national vaccination campaign and provided informed consent. Subjects (n = 16) were

298   26-59 years of age, both male and female, and healthy (demographic data presented in

299   Supplementary Table 1). Key exclusion criteria included a medical history of COVID-19. Sera and

300   blood samples were collected before the first administration of BNT162b2, three weeks after

301   the first dose (right before the second dose), and two weeks after the second dose. A high

302   percentage (56.3%) of individuals had been vaccinated with BCG in the past 12 months due to

303   the fact that many participants participated in parallel in a BCG-trial. One individual was

304   removed from the dataset after detecting high concentrations of antibodies against SARS-CoV-

305   2 N-antigen at baseline.

306

307   **Virus isolation and sequencing**

308   Viruses were isolated from diagnostic specimen at the department of Viroscience, Erasmus MC,

309   and subsequently sequenced to rule out additional mutations in the S protein: D614G

310   (BetaCoV/Munich/BavPat1/2020, European Virus Archive 026V-03883), B.1.1.7 (GISAID: hCov-

311   465 19/Netherlands/ZH-EMC-1148) and B.1.351 (GISAID: hCov-19/Netherlands/ZH-EMC-

312   1461). SARS-CoV-2 isolate BetaCoV/Munich/BavPat1/2020 (European Virus Archive 026V-

313   03883), was kindly provided by Prof. C. Drosten. SARS-CoV-2 Wuhan Hu-1 strain was kindly

314   provided by Prof. Heiner Schaal (Dusseldorf University, Germany). The B.1.1.7 and B.1.351

315   isolates were isolated from diagnostic specimens on Calu-3 lung adenocarcinoma cells for three

316   passages. Passage 3 BavPat1, B.1.1.7 and B.1.351 variants were used to infect Vero E6 cells at

317   an MOI of 0.01 in DMEM, high glucose (Thermo Fisher Scientific, USA, cat #11965092)

318   supplemented with 2% fetal bovine serum (Sigma-Aldrich, Germany, cat #F7524), 20 mM

319   HEPES (Thermo Fisher Scientific, USA, cat #15630056) and 50 U/mL penicillin-50 µg/mL

320   streptomycin (Thermo Fisher Scientific, USA, cat #15070063) at 37 °C in a humidified 5% CO_2

321   incubator. At 72 h post infection, the culture supernatant was centrifuged for 5 min at 1500 x

322   *g* and filtered through an 0.45 µM low protein binding filter (Sigma-Aldrich, Germany, cat

323   #SLHPR33RS). To further purify the viral stocks, the medium was transferred over an Amicon

324   Ultra-15 column with 100 kDa cutoff (Sigma-Aldrich, Germany, cat #UFC910008), which was

325   washed 3 times using Opti-MEM supplemented with GlutaMAX (Thermo Fisher Scientific, USA,

15

326   cat #51985034). Afterwards the concentrated virus on the filter was diluted back to the original

327   volume using Opti-MEM and the purified viral aliquots were stored at -80 °C. The infectious

328   viral titers were measured using plaque assays as described (Varghese et al., 2021) and stocks

329   were heat inactivated for 60 min at 56 °C for use in stimulation experiments.

330

331   **Measurement of antibody levels against RBD and Spike protein**

332   To measure the levels of antibodies against RBD and Spike protein, a fluorescent-bead-based

333   multiplex immunoassay (MIA) was developed as previously described by (Fröberg et al., 2021).

334   The stabilized pre-fusion conformation of the ectodomain of the Spike protein (amino acids 1

335   – 1,213) fused with the trimerization motif GCN4 (S-protein) and the receptor binding domain

336   of the S-protein (RBD) were each coupled to beads or microspheres with distinct fluorescence

337   excitation and emission spectra. Serum samples were diluted and incubated with the antigen-

338   coupled microspheres. Following incubation, the microspheres were washed and incubated

339   with phycoerythrin-conjugated goat anti-human, IgG, IgA and IgM. The data were acquired on

340   the Luminex FlexMap3D System. Mean Fluorescent Intensities (MFI) were converted to

341   arbitrary units (AU/ml) by interpolation from a log-5PL-parameter logistic standard curve and

342   log-log axis transformation, using Bioplex Manager 6.2 (Bio-Rad Laboratories) software.

343

344   **Plaque reduction neutralization assay**

345   A plaque reduction neutralization test (PRNT) was performed. Viruses used in the assay were

346   isolated from diagnostic specimen at the department of Viroscience, Erasmus MC, cultured

347   and subsequently sequenced to rule out additional mutations in the S protein: D614G

348   (GISAID: hCov-19/Netherlands/ZH-EMC-2498), B.1.1.7 (GISAID: hCov-19/Netherlands/ZH-

349   EMC-1148) and B.1.351 (GISAID: hCov-19/Netherlands/ZH-EMC-1461). Heat-inactivated sera

16

350   were 2-fold diluted in Dulbecco modified Eagle medium supplemented with NaHCO3, HEPES

351   buffer, penicillin, streptomycin, and 1% fetal bovine serum, starting at a dilution of 1:10 in 60

352   µL. We then added 60 µL of virus suspension (400 plaque-forming units) to each well and

353   incubated at 37°C for 1h. After 1 hour incubation, we transferred the mixtures on to Vero-E6

354   cells and incubated for 8 hours. After incubation, we fixed the cells with 10% formaldehyde

355   and stained the cells with polyclonal rabbit anti-SARS-CoV antibody (Sino Biological) and a

356   secondary peroxidase-labeled goat anti-rabbit IgG (Dako). We developed signal by using a

357   precipitate forming 3,3′,5,5′-tetramethylbenzidine substrate (True Blue; Kirkegaard and Perry

358   Laboratories) and counted the number of infected cells per well by using an ImmunoSpot

359   Image Analyzer (CTL Europe GmbH). The serum neutralization titer is the reciprocal of the

360   highest dilution resulting in an infection reduction of >50% (PRNT50). We considered a titer

361   >20 to be positive based on assay validation

362

Isolation of peripheral blood mononuclear cells

364   Blood samples from subjects were collected into EDTA-coated tubes (BD Bioscience, USA) and

365   used as the source of peripheral blood mononuclear cells (PBMCs) after sampling sera from

366   each individual. Blood is diluted 1:1 with PBS (1X) without Ca++, Mg++ (Westburg, The

367   Netherlands, cat #LO BE17-516F) and PBMCs were isolated via density gradient centrifuge

368   using Ficoll-PaqueTM-plus (VWR, The Netherlands, cat #17-1440-03P). The tubes used for the

369   isolation was specialized SepMate-50 tubes (Stem Cell Technologies, cat #85450) to ensure

370   better separation. Cells counts were determined via Sysmex XN-450 hematology analyzer.

371   Afterwards, PBMCs were frozen using Recovery Cell Culture Freezing (Thermo Fisher

372   Scientific, USA, cat #12648010) in the concentration of 15x106/mL.

373

374 **Simulation experiments**

375 The PBMCs were thawed and washed with 10mL Dutch modified RPMI 1640 medium (Roswell

376 Park Memorial Institute; Invitrogen, USA, cat # 22409031) containing 50 µg/mL Gentamicine

377 (Centrafarm, The Netherlands), 1 mM Sodium-Pyruvate (Thermo Fisher Scientific, USA, cat

378 #11360088), 2 mM Glutamax (Thermo Fisher Scientific, USA, cat #35050087) supplemented

379 with 10% Bovine Calf Serum (Fisher Scientific, USA, cat #11551831) twice, and afterwards the

380 cells were counted via Sysmex XN-450. PBMCs ($4x10^5$ cells/well) stimulated in sterile round

381 bottom 96-well tissue culture treated plates (VWR, The Netherlands, cat #734-2184) in Dutch

382 modified RPMI 1640 medium containing 50 µg/mL Gentamicine, 1 mM Sodium-Pyruvate, 2

383 mM Glutamax supplemented with 10% human pooled serum. Stimulations were done with

384 heat-inactivated SARS-CoV-2 Wuhan Hu-1 strain ($3.3x10^3$ TCID$_{50}$/mL), SARS-CoV-2 B.1.1.7

385 ($3.3x10^3$ TCID$_{50}$/mL), SARS-CoV-2 B.1.351 ($3.3x10^3$ TCID$_{50}$/mL), and SARS-CoV-2 Bavarian

386 ($3.3x10^3$ TCID$_{50}$/mL) variants, Influenza ($3.3x10^5$ TCID$_{50}$/mL), 10 µg/mL Poly I:C (Invivogen,

387 USA, cat #tlrl-pic), 3 µg/mL R848 (Invivogen, USA, cat #tlrl-r848), 10 ng/mL *E. coli* LPS, and 1 x

388 10^6 /mL *C. albicans.* The PBMCs were incubated with the stimulants for 24 hours to detect IL-

389 1β, TNF-α, IL-6, IL-1Ra and 7 days to detect IFN-γ. Supernatants were collected and stored in -

390 20°C. Secreted cytokine levels from supernatants were quantified by ELISA (IL-1β cat # DLB50,

391 TNF-α cat # STA00D, IL-6 cat # D6050, IL-1Ra cat # DRA00B, IFN-γ cat #DY285B, R&D Systems,

392 USA).

393

394 **Statistical analysis**

395 Graphpad Prism 8 was used for all statistical analyses. Outcomes between paired groups were

396 analyzed by Wilcoxon's matched-pairs signed-rank test. Three or more groups were

397 compared using Kruskal-Wallis Test - Dunnet's multiple comparison. P-value of less than 0.05

398   was considered statistically significant. Spearman correlation was used to determine

399   correlation between groups.

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