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Novel T cell interferon gamma release assay (IGRA) using spike recombinant protein for COVID19 vaccine response and Nucleocapsid for SARS-Cov2 response

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ABSTRACT

We explored the performance of a whole blood interferon gamma release assay (IGRA) based on the stimulation of SARS-Cov2-specific T cells by purified recombinant proteins. Twenty volunteers vaccinated with BNT162b2 were selected first for T cell response evaluation using an in-house IGRA, a commercial IGRA, and ELISpot showing a S2 > S1 poly-epitopic response. Next, 64 vaccinated and 103 non-vaccinated individuals were tested for humoral and T cell response (IGRA-Spike/−nucleocapsid recombinant proteins). Following the second vaccine injection, humoral (100%) and IGRA-Spike T cell (95.3%) responses took place irrespective of sex, age, and vaccine type. The humoral response declined first, followed by IGRA-Spike T cell response after the second vaccine injection. Altogether, this study confirms the utility of the IGRA-Spike/−nucleocapsid assay to complement serology in COVID19 vaccinated individuals and those who have recovered from SARS-Cov2.

1. Introduction

In the context of the SARS-Cov2 pandemic, diagnostic tests currently recommended focus on the detection of the virus, by rt-PCR or by antigenic tests, and on the detection of antibodies against Spike (S) and Nucleocapsid (Nuc) SARS-Cov2 proteins ([5,17]. In parallel, there has been a global effort to characterize the SARS-Cov2-specific T cell response based on ELISpot or flow cytometry, but these techniques suffer from limitations that preclude their generalization in a routine laboratory [18]. However, this gap can be overcome by using whole blood assays based on the evidence that the blood based interferon-gamma (IFN-γ) release assay (IGRA) is an attractive alternative to ELISpot, as demonstrated for tuberculosis [1]. Unfortunately, primary whole blood IGRA-based assays using exhaustive libraries of synthetic peptides for Spike and developed for routine use showed limited sensitivity in a COVID19 vaccinated volunteer population [8,14], which limits their clinical application.

Accordingly, in the present study, we have developed and next explored the performance of a new whole blood IGRA based assay on the stimulation of SARS-Cov2-specific T cells using purified recombinant proteins instead of overlapping peptides. For that, the *in vitro* response against S protein was evaluated in COVID19 vaccinated subjects using different technological platforms and approaches. Next, the humoral and SARS-Cov2-specific T cell responses were further explored against S and Nuc recombinant proteins in order to distinguish, respectively, the humoral and T cell responses in COVID19 vaccinated subjects and in those having recovered from asymptomatic SARS-Cov2 infections.

2. Material and methods

2.1. Individuals selected

From March to August 2021, 39 vaccinated staff members of the medical laboratory of the university hospital of Toulouse (CHU de Toulouse, Occitania, France) and 139 vaccinated/non vaccinated blood bank donors (EFS Toulouse, Occitania, France) were selected. Information collected included sex, age, vaccine types (BNT162b2/Pfizer-BioNTech, AZD1222/Astrazeneca-Oxford, and mRNA-1273/Moderna),

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and time from the second/last injection (Table 1). Exclusion criteria included a positive rt-PCR or antigen COVID19 test in the past and as inclusion criteria the declaration that they have never undergone any COVID-19 like infectious episode, which would have raised suspicion of SARS-Cov2 infection. When known individuals that were immunocompromised due to disease or treatment were not included in the study. All individuals were volunteers, have given their informed consent, and the study was conducted in accordance with the Declaration of Helsinki Principles.

2.2. Whole blood interferon gamma release assay (IGRA)

The blood samples were drawn into heparinized tubes (Becton Dickinson, Heidelberg, Germany), quickly routed to the laboratory as soon as possible (average reception time 4–6 h), shaken for homogenization, and whole blood used for IGRA analyses. In order to develop a new in-house IGRA assay, two protocols were performed: one for assay development in vaccinated individuals and another one for SARS-Cov2-specific T cell response evaluation in response to COVID19 vaccination. Regarding the in-house IGRA assay, the full-length Spike domain 1 for IGRA-S1, full-length Spike containing both S1 and S2 domains for IGRA-Spike and Nucleocapsid recombinant proteins for IGRA-Nuc were produced by INVIVOGEN (Toulouse, France) with a histidine tag and then purified on an affinity column. Protein sequences corresponded to those of the initial strain isolated in Wuhan, China [9]. The protein solutions sterilized by filtration were tested for endotoxins by functional tests using cells expressing human TLR2 and TLR4, and no reactivity was demonstrated. The protein solutions were diluted in RPMI (stock solutions: 1 μg/μL for assay development protocol and 0.1 μg/μL for protocol 2) and stored at −20 °C. The protein solutions were thawed *ad hoc* on the day of the test. Some tests were performed with aliquots that had undergone two freeze-thaw cycles without prejudice to the induction of the lymphocyte response.

For the assay development protocol, 20 vaccinated individuals were selected and tested both with the in-house IGRA and QuantiFERON SARS-CoV-2 Starter Pack (Qiagen, Valencia, CA; ref. 626715). The in-house IGRA assay includes the distribution of 1 mL of peripheral blood in 3 heparinized tubes with: (i) 20 μL of SARS-Cov2 S1 protein (20 μg/tube); (ii) 20 μL of RMPI (negative control); and (iii) 20 μL of phytohemagglutinin (PHA) used as a mitogen (40 μg/tube). Regarding the QuantiFERON SARS-Cov-2 assay, 1 mL of blood was also distributed in 4 tubes, which includes one tube (IGRA-QA) containing antigenic long peptide pools derived from Spike (for CD4+ T cell activation), one tube (IGRA-QB) with short and long peptide pools of Spike (for CD4+ and CD8+ T cell activation), and two control tubes, one positive control containing PHA and one negative control without stimulant.

For protocol 2, SARS-Cov2-specific T cell response evaluation, 103 non-vaccinated and 64 vaccinated individuals, including 12 individuals tested in protocol 1, 1 mL of blood was distributed in 4 tubes with: (i) 20 μL of SARS-Cov2 full-length Spike protein covering domains 1&2 (2 μg/tube); (ii) 2 μL of SARS-Cov2 Nuc protein (2 μg/tube); (iii) 20 μL of RMPI (negative control); and (iv) 20 μL of PHA (40 μg/mL). In some experiments, 1 mL of blood was distributed in an additional tube with 20 μL of SARS-Cov2 Spike domain 1 protein (2 μg/tube).

After an 18 to 24 h incubation at 37 °C, tubes were centrifuged, and the concentration of IFN-γ in supernatants was quantified by using the four-point standard curve of QuantiFERON Monitor ELISA technique (Qiagen), and results were expressed as international units (IU) of IFN-γ/mL. For analysis, data from the negative control tube was subtracted from the signal obtained after stimulation with peptides or recombinant proteins. QuantiFERON SARS-CoV-2 cut-off for positivity is proposed by the manufacturer at 0.150 IU IFN-γ/mL. The test is recorded as indeterminate when the negative control is >8 IU IFN-γ/mL or when the mitogen control <0.5 IU IFN-γ/mL, but such cases were not observed in this study.

Table 1
Description of the two cohorts used in the study. Of note, 12 individuals from the vaccinated cohort 1 were included in cohort 2.

	Vaccinated cohort 1 (n = 20)	Vaccinated cohort 2 (n = 64)	Non-vaccinated cohort 2 (n = 103)
Age in years (mean ± SEM)	55 ± 2	48 ± 2	38 ± 2
Sex (Male:Female)	6:14	37:27	35:68
Vaccine used: BNT162b2/ mRNA-1273/ AZD1222	20/0/0	50/8/6	–
Days from the second vaccine injection (mean ± SEM)	37 ± 2	104 ± 12	–

2.3. ELISPOT test

Within the 20 vaccinated individuals included in the assay development protocol, peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque density gradient centrifugation from 30 mL of EDTA-treated blood and RPMI washed cells stored frozen with 20% DMSO and fetal calf serum (FCS) in liquid nitrogen until the day of testing. PBMCs were thawed in a water bath (1–2 min at 37 °C) and left to recover overnight at 37 °C in RPMI culture medium with 20% FCS. Viability was assessed using acridine orange/propidium iodide (Cellometer Auto 2000, Nexcelom, MA) and 0.4 × 10^6 viable PBMCs were cultured for 36 h in duplicate wells with antigens in a final volume of 60 μL using plates, capture antibodies and detection reagents from the Diaclone kit for detecting IFN-γ (Diaclone, Besançon, France). The anti-SARS-Cov2 T cell response was assessed using 15-mers overlapping peptides (0.25 μg/mL) from 2 pools representing the S1 and the S2 domains (JPT-Peptide-Technologies, Berlin, Germany). Negative control wells lacked stimulating peptides, and positive control wells included CD3/CD28 stimulation (clones HIT3a and 28.2 respectively, 0.5 μg/mL each; BD Biosciences, Bedford, MA). Results are expressed as spot forming unit (SFU)/10^6 cells. The automated Immunospot S6 core reader and software (CTL Europe GmbH, Bonn, Germany) were used to count SFU using SmartCount™ and Autogate™ functions. According to the manufacturer's recommendations, specific responses were calculated after averaging duplicate wells and subtracting nonspecific responses (solvent without peptides).

2.4. Serological tests

The serological tests were carried out on serum. Serum tubes were centrifuged, and serum was then aliquoted and stored at −20 °C until analyzed. The total level of IgM/IgG/IgA antibodies to SARS-CoV-2 Spike mammalian cell-expressed recombinant protein was assessed in all individuals by means of the Wantai enzyme-linked immunosorbent assay (ELISA, Wantai Biological Pharmacy Enterprise, Beijing, China). ELISA total values are expressed in binding antibody units (BAU) per mL, using the WHO international standard NIBSC 20/136, and with an assigned cutoff >1 BAU/mL, as previously described [3] [4]. Selected individuals were further tested for IgG anti-Nuc antibodies (Abbott, Chicago, IL), and according to the manufacturer's guidance, a result of ≥1.4 (sample to calibrator [S/C] index) was considered positive.

2.5. Bioinformatics

Immune-Epitope Database and Analysis Resource (http://www.iedb.org/) was used in order to predict SARS-Cov2-specific T cell epitope positional frequency from the conserved SARS-Cov2 (5ID2697049) spike glycoprotein region (P0DTC2) (20). The request performed in September 2021 retrieved 231 epitopes and 414 assays from 23 references when using as selection criteria: human host, T cell IFN-γ release

assays, and any MHC restriction.

2.6. Statistics

Continuous data are described as mean ± standard error of the mean (SEM). Differences were analyzed using the *t*-test or the Mann-Whitney-Wilcoxon test when the normality was absent. Receiver operating curves (ROC) were generated to determine the area under the curve (AUC) and the optimal cut-off values were chosen using Youden's index. The Spearman rank correlation was used to compare techniques. Data were analyzed using GraphPad Prism 9.2 (La Jolla, CA), and a $p < 0.05$ considered significant.

3. Results

3.1. SARS Cov-2 specific response to spike S1 and S2 following last vaccination

In order to develop a whole blood IGRA to measure SARS-Cov-2 specific T cells in response to COVID-19 vaccination, the nature of the antigen is critical. Such an assertion is further supported by the observations that the S1 receptor-binding domain is less conserved as compared to the S2 fusion domain among betacoronaviruses (Fig. 1A, adapted from [7], and that T cell immunodominant regions are equally distributed between S1 and S2 domains (Fig. 1B). Accordingly, 20 volunteers having received 2 doses of BNT162b2 (Pfizer-BioNTech) were selected and tested after the second injection using: (i) an ELISpot assay following PBMC purification and stimulation with S1 and S2 overlapping peptides; (ii) whole blood IGRA stimulated with the S1 domain recombinant protein (IGRA-S1); and (iii) whole blood IGRA stimulated with long overlapping full-length S peptide pools (IGRA-QA, CD4 response), or long and short overlapping full-length S peptide pools (IGRA-QB, CD4/CD8 response).

Results from such analysis revealed: (i) a higher number of S2-specific T cell spots as compared to S1-specific T cell spots in the BNT162b2 vaccinated group tested by ELISpot was retrieved ($p = 0.03$, Fig. 2A). A strong correlation when comparing the S1 and S2 responses was further retrieved in the ELISpot assay ($r = 0.731$, $p = 0.0002$), which supports the concept that S1 and S2 T cell specific responses are combined in a poly-epitopic response; (ii) a similar magnitude for whole blood IGRA response when comparing S1 recombinant protein with long ± short overlapping S peptides in IGRA-QB (Fig. 2B), while a higher magnitude was reported when comparing recombinant protein S1 to long overlapping S peptides in IGRA-QA (CD4 response only); and furthermore (iii) a strong correlation between whole blood IGRA and ELISpot when using S2 ($r = 0.593$ with S1 to 0.773 with IGRA-Q1; $p < 0.03$ for all) was retrieved, which showed less contrast when using as reference ELISpot with S1 protein ($r = 0.321$ with IGRA-S1 to 0.538 with IGRA-QB; nonsignificant for IGRA-S1 protein and $p < 0.05$ for IGRA-QA/B), or combination of S1 and S2 in ELISpot (Fig. 2D).

Altogether this prompts us to consider that testing of the SARS-Cov2 spike-specific T cell response needs to evaluate both S1 and S2 epitopes. As reported in Fig. 3, after the last dose of COVID19 vaccine, IGRA-S1 protein results were similar when using 2 μg/tube instead of 20 μg/

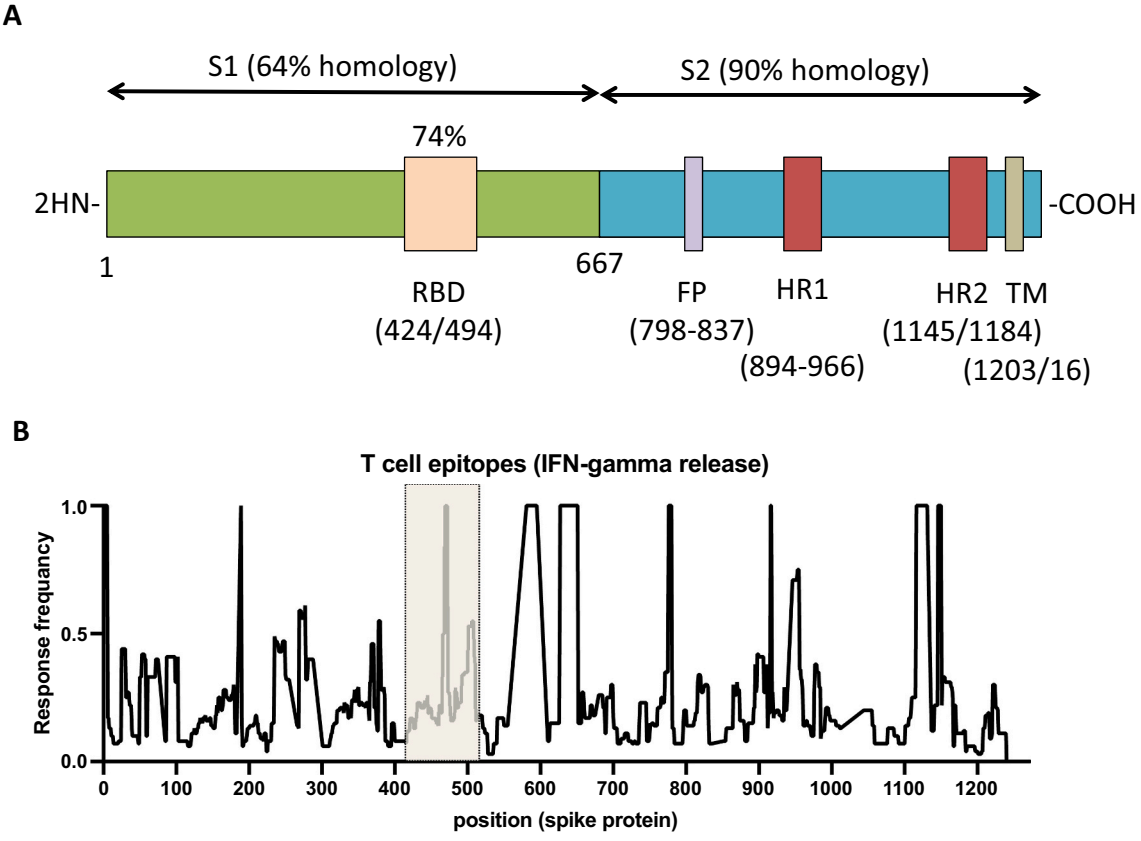

Fig. 1. SARS-Cov2 Spike protein sequence analysis. A- Protein sequence alignment between SARS-Cov2 and beta-coronaviruses reveals that most of the divergence is located in domain S1 although the receptor binding domain (RBD) is well conserved. Domain S2 and its subdomains are well conserved (>90%): fusion peptide region (FP), heptad-repeat (HR) 1 and 2 domains, and the transmembrane domain (TM). Adapted from [7]. **B-** The Immune-Epitope Database and Analysis Resource (http://www.iedb.org/) tool was used to identify potential antigenic regions across the SARS-Cov2 Spike proteome. For visualization, the response frequency score is established for each peptide position used in a CD4/CD8 T cell IFN-γ release assay. The RBD part of spike is indicated through its role in viral cell entry *via* interaction with the angiotensin-converting enzyme 2 (ACE2) receptor on host cells, its recognition by neutralizing antibodies, and the presence of variants of concern (VOC).

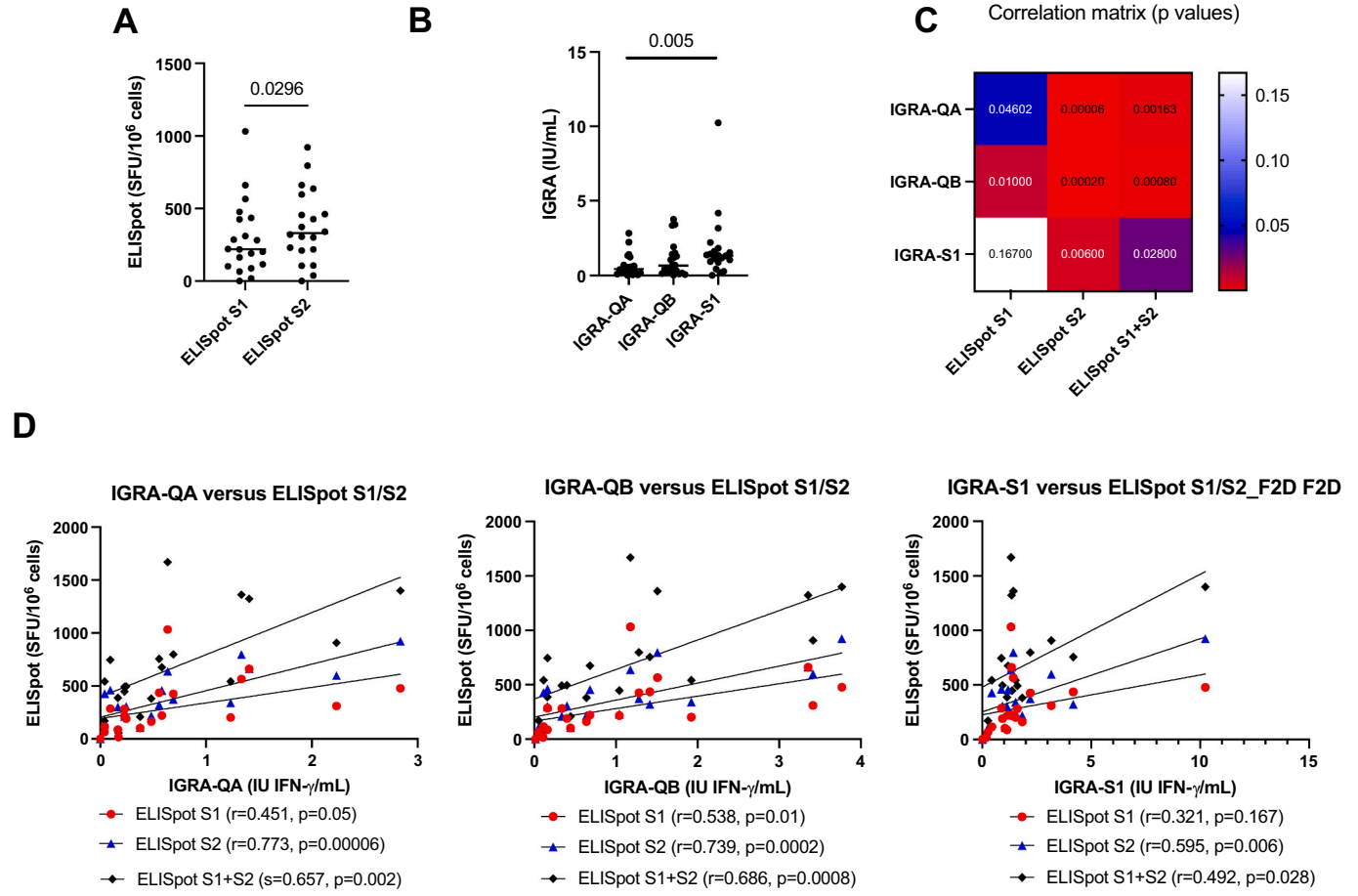

Fig. 2. SARS-Cov2 specific T cell response to spike using different platforms in 20 volunteers following thesecond injection of**BNT162b2 vaccination boost. A-***Ex-vivo*** interferon gamma (IFN-γ) ELISpot results showing T cell response to Spike domain 1 (S1) and domain 2 (S2) overlapping peptides. The results are expressed as spot forming unit (SFU)/10^6 cells of peripheral blood mononuclear cells (PBMCs). **B-** Whole blood IFN-γ release assay (IGRA) response to a peptide pool derived from spike (IGRA-QA: long peptides for CD4 T cell stimulation; and IGRA-QB long and short peptides for CD4 and CD8 T cell stimulation; QuantiFERON SARS-Cov2) or from the S1 recombinant protein (in house IGRA-S1). **C:** Correlation map between ELISpot S1, ELISpot S2, and ELISpot S1 + S2 responses with IGRA-QA, IGRA-QB and in-house IGRA-S1. **D:** Correlation between ELISpot (S1, S2, and S1 + S2) and IGRA (IGRA-QA: left; IGRA-QB: center; and IGRA-S1: reight). Spearman's rho(r) and *p* values <0.05 are indicated when significant.

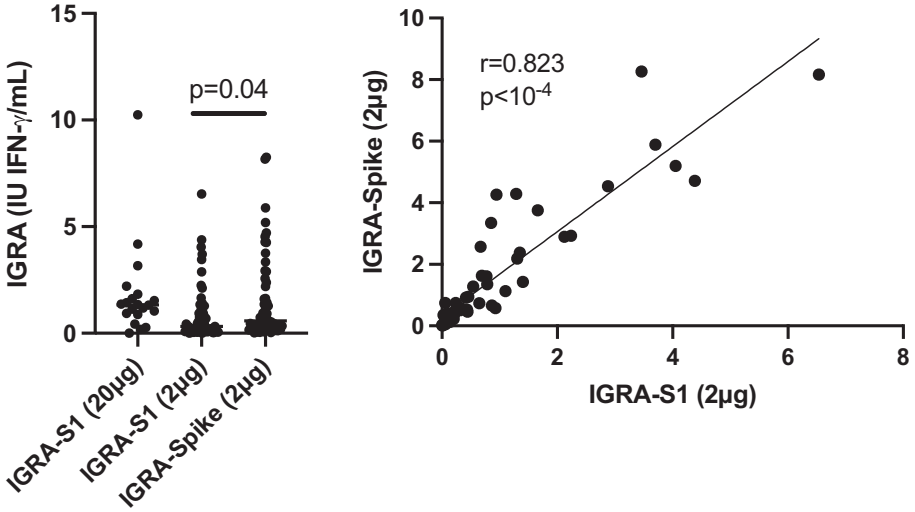

Fig. 3. Whole blood interferon gamma release assay (IGRA) response to Spike domain 1 (S1) and full-length protein (Spike) following thesecond injection **of COVID19 vaccine. A-** IGRA-S1 and IGRA-Spike performances according to the final concentration of the recombinant protein. IGRA-S1 (20 μg/tube, $n = 20$), IGRA-S1 (2 μg/tube, $n = 57$), IGRA-Spike (2 μg/tube, n = 57). **B-** Correlation between IGRA-S1 (2 μg/tube) and IGRA-Spike (2 μg/tube). Spearman's rho(r) and p values <0.05 are indicated when significant.

tube. Higher IGRA values were obtained when using full-length Spike that possess two-fold more immunodominant T cell regions than S1 that is restricted to domain 1 of Spike ($p = 0.04$), and both IGRA-S1 and IGRA-Spike results were strongly correlated ($r = 0.823$, $p < 10^{-4}$). Accordingly, 2 μg/tube of the recombinant protein and full-length spike instead of S1 were next selected.

3.2. SARS-Cov2 specific T cell and humoral responses in vaccinated individuals

In the context of COVID19 vaccination, both T cell and humoral responses to SARS-Cov2 spike are engaged. Accordingly, 103 non-vaccinated individuals were compared with 64 individuals having received the second dose of COVID19 vaccine (BNT162b2 $n = 50$, mRNA-1273 $n = 8$, and AZD1222 $n = 6$). As expected, and presented in Fig. 4A–C, ELISA anti-Spike total immunoglobulin levels (3201 ± 472 BAU/mL in vaccinated *versus* 5.4 ± 4.2 BAU/mL in non-vaccinated; $p < 10^{-4}$) and IGRA-Spike response to the recombinant protein (1.50 ± 0.24 IU IFN-γ/mL in vaccinated *versus* 0.04 ± 0.02 IU IFN-γ/mL in non-vaccinated; $p < 10^{-4}$) were increased in the vaccinated group. When using the whole SARS-Cov2 Nuc recombinant protein, not present in the COVID19 vaccines, IGRA-Nuc response was similar between vaccinated and non-vaccinated individuals ($p = 0.184$).

Within the non-vaccinated group and although the introduction of restrictive criteria to exclude volunteers having tested/declared to have encountered SARS-Cov2, a possible past and asymptomatic infection with SARS-Cov2 may be suspected (Table 2). This is based on the

Table 2
Individual repartition according to the response to anti-SARS-Cov2-spike total antibodies (ELISA), IFN-γ release assay (IGRA) against Spike (S) or nucleoplasmin (Nuc) according to the vaccination status. Positive cut-offs are indicated.

Anti-Spike ELISA (>1.0 BAU/mL)	IGRA-S (>0.04 IU IFN-γ/mL)	IGRA-Nuc (>0.04 IU IFN-γ/mL)	Vaccinated	Non-vaccinated
+	+	+	5	3
+	+	−	56	1
+	−	−	3	0
−	+	−	0	5
−	−	+	0	1
−	−	−	0	93

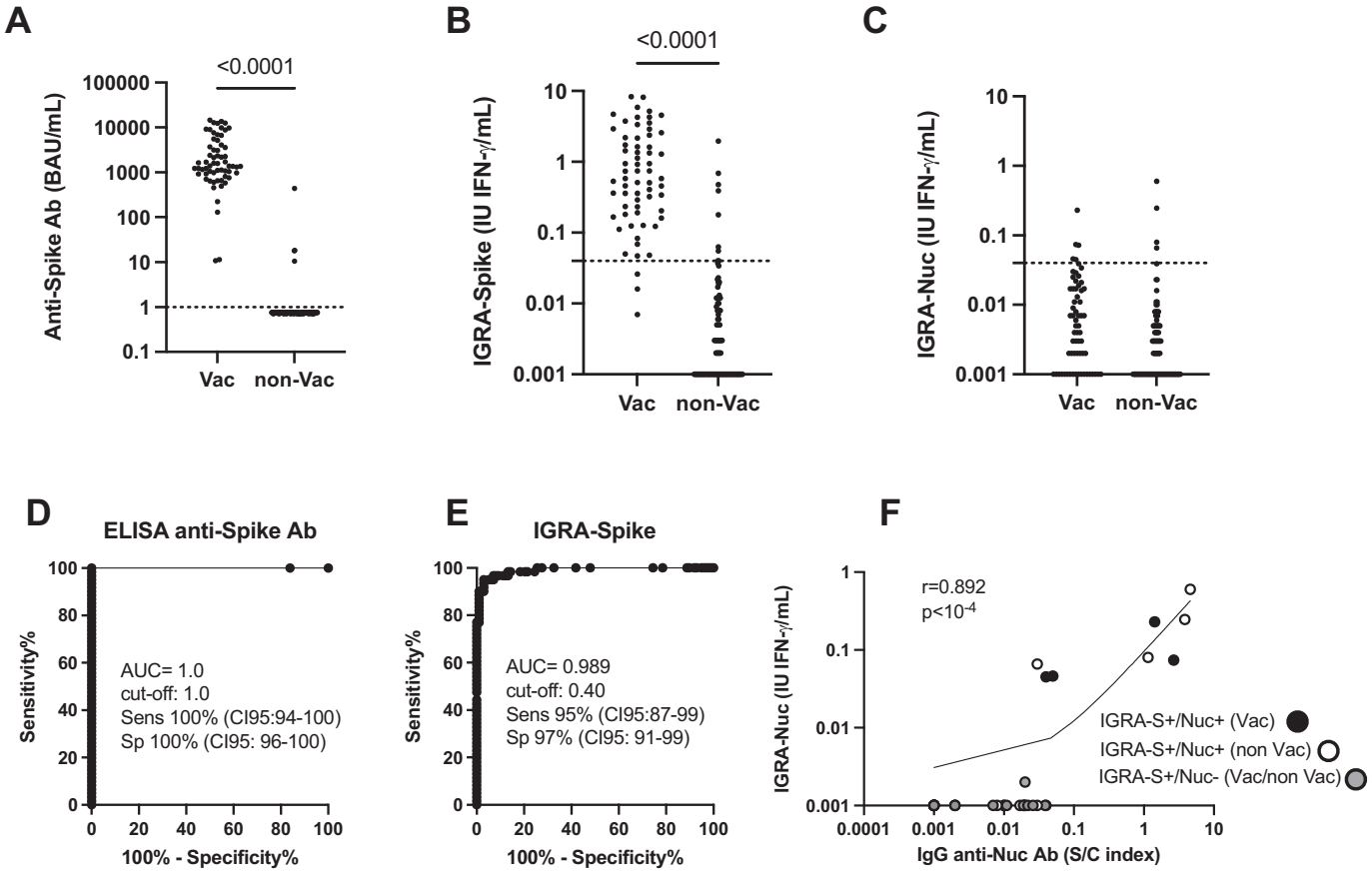

Fig. 4. SARS-Cov2 humoral and T cell responses in vaccinated individuals (Vac, $n = 64$) as compared to non-vaccinated volunteers (non-Vac, $n = 103$). A- Anti-SARS-Cov2 Spike total IgM/IgG/IgA antibody (Ab) titers (BAU/mL) by ELISA. **B-** Whole blood IFN-γ release assay (IGRA) response to the full-length Spike recombinant protein (IGRA-Spike). **C-** Whole blood IGRA response to the nucleocapsid recombinant protein (IGRA-Nuc). **D/E:** Receiver Operating Characteristic curve to establish anti-Spike Ab and IGRA-Spike cut-offs at which sensitivity and specificity are optimal (Youden's index). Due to the elevated rate of individuals having recovered from asymptomatic SARS-Cov2 infection, populations were corrected to determine cut-off for: (i) anti-SARS-Cov2 Spike total IgM/IgG/IgA antibody (non-vaccinated individuals with IGRA-Spike and/or IGRA-Nuc >0.040 IU IFN-γ/mL were removed); (ii) IGRA-Spike (vaccinated and non-vaccinated individuals with IGRA-Nuc >0.04 IU IFN-γ/mL plus non-vaccinated individuals with anti-SARS-Cov2 antibodies >1.0 BAU/mL were removed); (iii) IGRA-Nuc (non-vaccinated with IGRA-Spike >0.04 IU IFN-γ/mL and anti-SARS-Cov2 antibodies >1.0 BAU/mL were removed). **F:** Correlation between IgG anti-Spike Ab and IGRA-Nuc in individuals positive for IGRA-Nuc among vaccinated (black circle, $n = 4$) and non-vaccinated (white circle, n = 4); positive for IGRA-Spike while IGRA-Nuc negative among vaccinated ($n = 23$) and non-vaccinated (n = 5), grey circles. For graphical purposes, values at 0.001 or lower were fixed at 0.001. Cut-off (dot line), area under the curve (AUC), cut-off, optimal sensitivity, and specificity, 95% confidence interval (CI95), Spearman's rho(r) and p values <0.05 are indicated when significant.

observations that 4/103 (3.9%) in the non-vaccinated group were positive for anti-SARS-Cov2-spike antibodies, and some of them presented elevated IFN-γ levels with IGRA-S and/or IGRA-Nuc. Such observation is not surprising as a report has estimated that 2–5% of the population had encountered SARS-Cov2 in the French region of Occitania after the first lockdown, from March 17th/May 11th, 2020 [5]. Accordingly, and in order to fix the cut-off for positivity, individuals suspected to have encountered SARS-Cov-2 were removed from the ROC analysis (see Fig. 4D–E for details). The Youden's index was used next to establish the cut-offs at which the sensitivity and specificity were maximal corresponding to 0.040 IU IFN-γ/mL for IGRA-Spike (sensitivity 95%; specificity 97%) and to 1.0 BAU/mL for the anti-Spike antibody ELISA (sensitivity 100%; specificity 100%).

Next, at established cut-offs, the vaccinated subgroup was defined by the detection of anti-Spike antibodies (100%) and an IGRA-Spike protein T cell response (95.3%); the two parameters were not correlated ($r = 0.18$; non significant). Using the IGRA-Spike protein cut-off fixed at 0.04 IU IFN-γ/ml, an IGRA-Nuc protein response was retrieved for 5/64 (7.8%) vaccinated individuals, which suggested a previous infection with SARS-Cov2 among the vaccinated subgroup. Within the non-vaccinated subgroup, most of the individuals were negative for the three tests (92/103, 89.3%). A positive IGRA-Spike±Nuc T cell response was retrieved for 10/103 (9.7%) non-vaccinated individuals and, among them, anti-SARS-Cov2 Spike antibodies were retrieved for 3 of them. Discordant IGRA results (vaccinated: IGRA-Nuc positive and non-vaccinated: IGRA-S and/or IGRA-Nuc positive) plus 23 non-selected vaccinated individuals were further tested for IgG anti-Nuc antibodies (Fig. 4F), a strong correlation ($r = 0.892$, $p < 10^{-4}$) was retrieved between the IGRA-Nuc assay and anti-SARS-Cov2-Nuc antibodies. Altogether, this supports that T cell response analysis is performant to evaluate possible occurrence of a previous SARS-Cov2 infection.

3.3. Characteristics of the humoral and T cell response in vaccinated individuals

Finally, within the vaccinated subgroups the influence of sex, age > 50 years, type of vaccine, and delay from the second injection were studied further. As presented in Fig. 5, anti-SARS-Cov2 antibodies declined progressively with lower levels obtained after 100 days following the last vaccination injection ($p = 0.004$: 0–29 days *vs* >100 days; $p = 0.04$: 0–30 days *vs* >100 days), which is in agreement with a previous observation from our group [3]. In comparison, the decline in the IGRA-Spike response was delayed ($p = 0.02$: 0–29 days *vs* >100 days). Humoral and T cell responses were independent from sex, age, and the type of vaccine used.

4. Conclusion

In this study we have developed a new whole blood IGRA test based on the use of SARS-Cov2 recombinant proteins for both Spike and Nucleocapsid in a healthy population vaccinated or not for COVID19, mainly with BNT162b2. We have first confirmed that whole blood IGRA

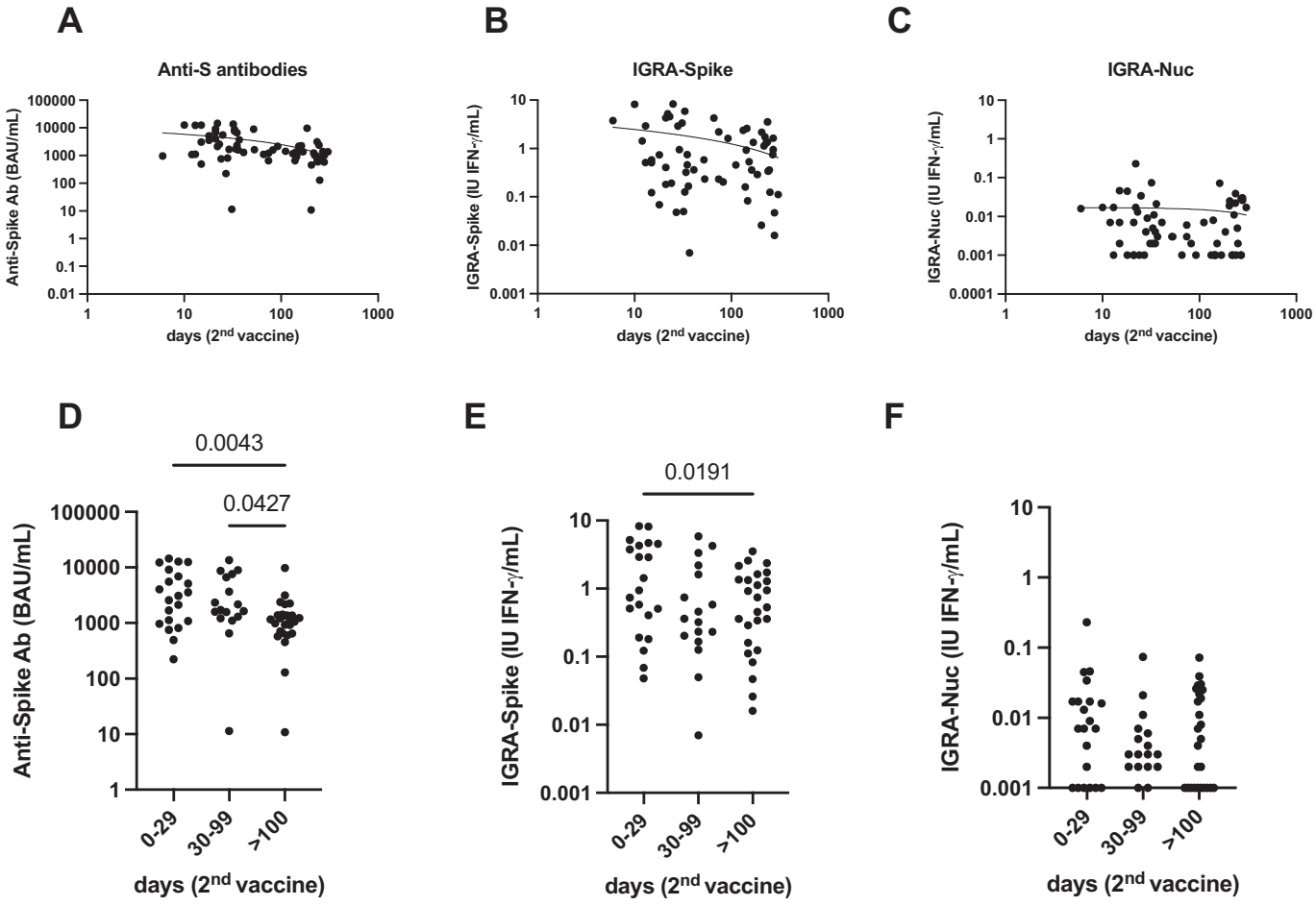

Fig. 5. SARS-Cov2 humoral and T cell kinetics and responses in volunteers tested < 30 days ($n = 21$), between 30 and 100 days ($n = 17$) and > 100 days ($n = 26$) after the second injection of COVID19 vaccine. A/D- Anti-SARS-Cov2 antibody (Ab) response. **B/E-** IFN-γ release assay (IGRA) response to the full-length Spike recombinant protein. **C/F-** IGRA to the nucleocapsid (Nuc) recombinant protein. For graphical purposes, values at 0.001 or lower were fixed at 0.001. Individual results and p values <0.05 are presented when significant.

can be used as a surrogate of the ELISpot assay, and such an assertion is reinforced when the S2 domain of Spike is included in the assay. Second, individuals develop a strong humoral and T cell Spike response after the second COVID19 vaccine injection, and with a humoral response that declines first. Third, T cell response analysis (against Spike and Nuc proteins) performed better than humoral analysis to measure SARS-Cov2 transmission rate in individual and population studies.

Cellular immunity stimulated by COVID19 vaccine is characterized by both antibodies against the Spike protein and the activation of Spike specific CD4 T cells with a TH1 profile. BNT162b2 additionally induces a strong CD8 T cell response after primary and booster vaccination [10,13]. T cell epitopes are distributed all over the Spike protein, which was retrieved when we performed the Immune-Epitope Data-base analysis. In addition, it was reported that S1 T cell epitopes overlap with B cell epitopes, neutralizing antibody binding sites, and variants with a significant impact on transmissibility, severity and/or immunity (referred to as variants of concern [VOC]), while S2 T cell epitopes are more conserved and shared with other coronaviruses [2,7]. Both S1 and S2 domains are recognized in a poly-epitopic T cell response following the second injection of BNT162b2 but with a predominant T cell response to S2 as compared to S1, which was retrieved in our study and reported previously [13,19]. When regarding associations between the humoral and cellular response, both Spike specific CD4 T cell and CD8 response were correlated with S1-binding specific IgG in the Sahin *et al* study, an observation not confirmed in our study when testing total IgM/IgG/IgA antibodies instead of IgG anti-SARS-CoV-2 Spike antibodies. By contrast the correlation between specific T cell response to Nuc (IGRA-Nuc) and IgG anti-SARS-Cov2 Nuc antibodies was reported in our study.

Determining the cut-off for IGRA is challenging. Therefore, the provider has established the cut-off in the QuantiFERON assay at 0.350 IU IFN-γ/mL for mycobacterium tuberculosis, at 0.200 IU IFN-γ/mL for cytomegalovirus, and proposed 0.150 IU IFN-γ/mL for SARS-Cov2. When using the QuantiFERON SARS-Cov2 assay in vaccinated healthy individuals following the second injection, the prevalence ranged from 44% when using mRNA-1273 to 80% with BNT162b2 [8,14], which supports the need to optimize the IGRA assay. This can be done, as performed in our study, by taking into consideration several parameters: (i) the use of recombinant proteins instead of overlapping peptides, making it possible to avoid presentation to T cells by antigen presenting cells (APC) of overlapping and degradation peptides; (ii) a large population of non-vaccinated individuals; and (iii) the detection of a specific T cell response distinct from Spike, such as directed against the nucleocapsid, to distinguish vaccination from recent infections and or pre-existing immunity to seasonal human beta-coronaviridae [11]. For IGRA-Nuc used to explore previous infections, elevated IFN-γ levels were retrieved within the vaccinated (7.9%) and the non-vaccinated (3.9%) groups. Altogether this allows fixing the cut-off at 0.040 IU IFN-γ/mL (sensitivity 98%; specificity 94%) as compared to 0.150 IU IFN-γ/mL with the QuantiFERON SARS-Cov2 assay. Moreover, the use of PHA as a positive control for IGRA further allows assessment of T cell functionality that could be impaired in the case of leukopenia, in which there has been use of immunosuppressants including steroids, or when tubes are improperly shaken or delayed (>2 days). We did not find that COVID19 vaccine generates an inadequate IFN-γ response to PHA, while patients with severe COVID19 disease have been described to have a 6-fold reduction in IFN-γ levels when stimulated with PHA [16]. As such, an indeterminate IGRA-Covid assay has to be considered with caution in patients with active COVID19.

Assessing the SARS-Cov2 T cell response, in addition to humoral immunity, appears important in several conditions. First, the presence of SARS-Cov2-specific T cell response may indicate a lower risk of severe disease in vaccinated individuals with antibody deficiency [5]. Second, to test an inadequate SARS-Cov2-specific T cell response in those individuals presenting persistent infections with SARS-Cov2 and/or long COVID19 syndrome [12]. Third, COVID19 vaccine T cell and humoral responses are not equal against mutated variants of SARS-Cov2 with a more resilient T cell response reported [15]. Such observations rely on the fact that VOC and neutralizing antibody response occurs predominantly in the less conserved Spike domain (S1) that mediates attachment to ACE2, as compared to the highly conserved S2 part implicated in cellular fusion and the main T cell response [19]. The predominant T cell response to S2 was further confirmed in our study when testing vaccinated individuals with two-fold more clones directed to S2 than S1 peptides in the ELISpot assay and when comparing the IGRA response between S1 protein fragment and full-lenght S protein.

SARS-Cov2 transmission rate determination as well as assessing any previous asymptomatic SARS-Cov2 infection are challenging due to the transient humoral response [3]. Accordingly, it has been proposed that the T cell-response represents a more sensitive indicator as compared to antibody assays [6]. Such an assertion is supported in our study with the observations that less than half of the individuals with discordant IGRA responses within non-vaccinated individuals possess anti-Spike antibodies, and that among vaccinated individuals anti-Spike antibody titers start to decline before the IGRA-Spike T cell response. However, it could not be excluded that a T cell response against cross-reacting antigens may also occur, including with common cold coronavirus based on homology over 90% with both S2 domain and nucleocapsid [7].

This study has limitations including a monocentric evaluation and the recruitment of volunteers that do not reflect interfering factors encountered in patients such as ongoing disease, immunosuppression, infections… Moreover, IGRA techniques are influenced by preanalytical considerations (*e.g.*, transport and incubation times, shaking), leucopenia, immunocompetent status (innate, acquired including with immunosuppressive treatments). This question has been explored in pre-pandemic healthy individuals showing a cross-reactive memory T cell response restricted to S1/S2, suggesting that IGRA-Nuc response predicts predominantly a previous SARS-Cov2 infection as supported by our report of a correlation between IGRA-Nuc and anti-SARS-Cov2 Nuc antibodies [11].

In conclusion and in contrast to laboratory research techniques (*e.g.* ELISpot, FACS), the in-house whole blood IGRA-COVID19 developed in this study is easy to perform, applicable to a large number of samples, with results in 48 h, and therefore is particularly suitable for the routine exploration in terms of specificity and sensitivity of the SARS-Cov2-specific T cell response in vaccinated individuals (IGRA-S) as well as for those having recovered from SARS-Cov2 infection (IGRA-Nuc). As a routine perspective, whole blood IGRA-COVID19 assays may become a valuable tool for vulnerable groups at risk, such as immunocompromised individuals that failed to make a suitable antibody and/or cellular response, and to evaluate long term-term perturbation of the peripheral immune system when individuals have not recovered months after acute SARS-Cov2 infections. For that, T cell explorations have to be completed with the analysis of the antibody response as cellular and humoral responses have both a distinct role in controlling viral infection. Another important point, not answered yet regarding IGRA-COVID19, is to establish the level at which T cell protection is effective.

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Declaration of Competing Interest

None.

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