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SARS-CoV-2 infection long time after full vaccination is related to a lack of neutralizing antibodies

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ABSTRACT

SARS-CoV-2 infections after COVID-19 vaccination are not unexpected, but those occurring more than 14 days after second vaccine dose need to be investigated. We describe a well-characterized infection which occurred almost 2 months after full vaccination, and provide the evidence of a link with a lack of anti-SARS-CoV-2 neutralizing antibodies.

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Dear Editor,

Vaccines are a critical tool to bring under control the COVID-19 pandemic, and have been developed and approved at unprecedented rates, especially mRNA-based vaccines. Early real-life experience suggests more than 90% protection against symptomatic illness, severe disease and hospitalization in the period starting from 7 days after the second dose (Dagan *et al.*, 2021; Thompson *et al.*, 2021), but also effectiveness in preventing asymptomatic infections and thus transmission (Tande *et al.*, 2021). However, the overall picture has become more complex since the emergence of numerous SARS-CoV-2 variants worldwide (Boehm *et al.*, 2021). Infections occurring after full immunization need to be well understood in order to adapt prevention strategies.

We here report a well-characterized infection which occurred almost 2 months after full vaccination, and provide the evidence of a link with a lack of anti-SARS-CoV-2 neutralizing antibodies.

A 59-year-old French male healthcare worker was vaccinated, as recommended by national guidelines, with Pfizer mRNA SARS-CoV-2 vaccine (Comirnaty). He received the first and second doses on January 7 and January 28, 2021, respectively. He had no history of previous COVID-19, and had no underlying conditions. On March 25, his non-vaccinated wife who experienced a loss of taste and smell, went to a pharmacy for a COVID-19 rapid antigenic test which was found

positive. Later that day, both of them underwent a laboratory-based molecular SARS-CoV-2 testing on nasopharyngeal specimens. A positive result was found for the 2 samples. A serum sample was additionally collected from the vaccinated healthcare worker on March 26, 2021 for antibody testing.

At the time of sampling, the patient had a runny nose, and developed later other mild symptoms such as sore throat, dry cough, anosmia and ageusia. He fully recovered after 10 days.

SARS-CoV-2 RT-PCR was performed using the Simplexa™ COVID-19 Direct kit on the Liaison MDX instrument (Diasorin®). This rapid molecular assay targets two different regions of the SARS-CoV-2 genome, ORF 1ab and Spike (S). The Ct values observed for the patient's sample were 13 and 12 for ORF 1ab and S genes, respectively. The viral whole genome sequencing was performed using the Illumina COVIDSeq Test on the NextSeq 550 System, and identified a SARS-CoV-2 B.1.1.7 lineage (WHO VOC "Alpha") with no additional mutations of interest in the S region. The viral sequences corresponding to both patient and wife's isolates have been submitted to GISAID (accession references EPI_ISL_1910906 and EPI_ISL_1910905).

Anti-SARS-CoV-2 antibodies were investigated using the WANTAI SARS-CoV-2 Ab and IgM ELISA assays (Eurobio Scientific) detecting anti-S total and IgM antibodies respectively. The result was positive for total antibodies (index at 9.7, cut-off: 1.1), and negative for IgM. In addition, anti-S IgG antibodies were quantified using the Anti-SARS-CoV-2 QuantiVac ELISA (IgG) assay (Euroimmun), and the IgG

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concentration was evaluated to 379 binding antibody units per milliliter (BAU/mL), with a positivity cut-off set at 35.2 BAU/mL.

Neutralizing antibodies were investigated using a live virus neutralization assay. Briefly, serial 2-fold dilutions of the serum (starting from 1:10 up to 1:2560) were incubated at 37°C for 1 hour with 100 TCID₅₀ of virus, and mixes were added to Vero E6 cell monolayers in a 96-well plate. The cytopathic effect was recorded after 3 days, and the serum virus neutralization titer (VNT₅₀) was defined as the reciprocal value of the highest dilution that showed at least 50% protection of cells. A sample with a titer ≥ 20 was defined as positive.

The patient's serum was tested against three different virus isolates: 20A EU2, Alpha and Beta variants (GISAID accession references EPI_ISL_1653927, EPI_ISL_1653931, EPI_ISL_1653932 respectively). Both heat-inactivated (56°C for 30 minute) and non-inactivated sera were tested. No neutralizing activity was detected in patient's sample, and VNT₅₀ was < 20 in all conditions. Nevertheless, a serum sample collected 2 months after the infection, showed a neutralizing activity with a VNT₅₀ at 80 against the Alpha variant.

Evidence from clinical trials and real-life cohorts has shown the effectiveness of COVID-19 vaccines against both asymptomatic and symptomatic infections, although infections after vaccination are not unexpected since vaccines are not 100% effective. However they seem to be mostly asymptomatic and occur early after partial of full vaccination, likely in patients exposed before immunity development (Dagan et al., 2021; Keehner et al., 2021; Tande et al., 2021; Thompson et al., 2021). In France, the vaccine effectiveness 7 days after the second dose of mRNA vaccine was recently estimated at 86% against COVID-19 with the B.1.1.7 lineage (Charmet et al., 2021). Cases of particular interest are patients who get infected long time after full immunization (at least 14 days after the second vaccine dose). These cases classified as COVID-19 vaccine breakthrough infections, need to be investigated in order to improve prevention strategies. The failure could be related to the susceptibility of the SARS-CoV-2 variant causing the infection, or to the host response. Immune response to vaccination is complex and multifactorial including antibodies as well as cellular immunity, and can be variable from one individual to another. In the case presented here, the patient was infected with the widely circulating B.1.1.7 lineage (Alpha variant) whose infection was reported to be preventable by the Pfizer mRNA SARS-CoV-2 vaccine-induced antibodies (Planas et al., 2021). The breakthrough was therefore likely due to the host response. The subject might fall in the fraction of the population without optimal response.

A seroconversion had been achieved, confirming the vaccination. However significant IgG levels were not correlated with effective neutralizing activity. This lack of neutralization could explain the high viral replication at the time of diagnosis. Whether this impaired response was related to the administered product or to the subject's immune system, remains an open question in a patient with no underlying conditions and no ongoing treatments (a serum neutralizing activity was observed after natural infection). However, it cannot be excluded that the vaccination contributed to the mild clinical features experienced by the patient, protecting him from severe disease.

Detailed studies investigating immune response in vaccine breakthrough cases are needed.

Our observation is in agreement with a recent case-control study in Israel which found low antibody levels in vaccine breakthrough cases as compared to controls (Bergwerk et al., 2021).

Antibody levels obtained with routine ELISA quantitative assays should be interpreted with caution, because they might not reflect serum neutralizing activity, as observed in this report. The correlation between these tests and seroneutralization assays need to be studied in sera from both patients with confirmed SARS-CoV-2 past infection, and vaccinated people.

In conclusion, the investigation of COVID-19 vaccine breakthrough cases should also consider the evaluation of the host response, including testing for serum neutralizing activity.

Authors' contributions

EKA: Conceptualization; Investigation; Methodology; Writing - original draft. OG: Investigation; Writing - review & editing. AG: Methodology; Writing - review & editing. CT: Methodology; Writing - review & editing. ML: Methodology; Writing - review & editing. LB: Methodology; Writing - review & editing. IE: Methodology; Writing - review & editing. DH: Supervision; Writing - review & editing.

Declaration of competing interest

The authors report no conflicts of interest relevant to this article.

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