

Vaccine breakthrough infections with SARS-CoV-2 Alpha mirror mutations in Delta Plus, Iota, and Omicron

Brenda Martínez-González,¹ Lucía Vázquez-Sirvent,¹ María E. Soria,^{1,2} Pablo Mínguez,^{3,4,5} Llanos Salar-Vidal,¹ Carlos García-Crespo,² Isabel Gallego,^{2,6} Ana I. de Ávila,² Carlos Llorens,⁷ Beatriz Soriano,⁷ Ricardo Ramos-Ruiz,⁸ Jaime Esteban,¹ Ricardo Fernandez-Roblas,¹ Ignacio Gadea,¹ Carmen Ayuso,^{3,4} Javier Ruíz-Hornillos,^{9,10,11} Concepción Pérez-Jorge,¹ Esteban Domingo,^{2,6} and Celia Perales^{1,6,12}

¹Department of Clinical Microbiology, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz University Hospital, Universidad Autónoma de Madrid (IIS-FJD, UAM), Madrid, Spain. ²Centro de Biología Molecular “Severo Ochoa” (CBMSO), Consejo Superior de Investigaciones Científicas (CSIC-UAM), Campus de Cantoblanco, Madrid, Spain. ³Department of Genetics and Genomics, IIS-FJD, UAM, Madrid, Spain. ⁴Centre for Biomedical Network Research on Rare Diseases (CIBERER), Instituto de Salud Carlos III, Madrid, Spain. ⁵Bioinformatics Unit, IIS-FJD, UAM, Madrid, Spain. ⁶Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Instituto de Salud Carlos III, Madrid, Spain. ⁷Biotechvana, “Scientific Park,” Universidad de Valencia, Valencia, Spain. ⁸Unidad de Genómica, “Scientific Park of Madrid,” Campus de Cantoblanco, Madrid, Spain. ⁹Allergy Unit, Hospital Infanta Elena, Valdemoro, Madrid, Spain. ¹⁰IIS-FJD, UAM, Madrid, Spain. ¹¹Faculty of Medicine, Universidad Francisco de Vitoria, Madrid, Spain. ¹²Department of Molecular and Cell Biology, Centro Nacional de Biotecnología (CNB), CSIC, Campus de Cantoblanco, Madrid, Spain.

Replication of SARS-CoV-2 in the human population is defined by distributions of mutants that are present at different frequencies within the infected host and can be detected by ultra-deep sequencing techniques. In this study, we examined the SARS-CoV-2 mutant spectra of amplicons from the spike-coding (S-coding) region of 5 nasopharyngeal isolates derived from patients with vaccine breakthrough. Interestingly, all patients became infected with the Alpha variant, but amino acid substitutions that correspond to the Delta Plus, Iota, and Omicron variants were present in the mutant spectra of the resident virus. Deep sequencing analysis of SARS-CoV-2 from patients with vaccine breakthrough revealed a rich reservoir of mutant types and may also identify tolerated substitutions that can be represented in epidemiologically dominant variants.

Introduction

SARS-CoV-2 continues its diversification worldwide, and a new variant termed Omicron (B.1.1.529), carrying a large number of mutations, was recently described in South Africa and classified as a potential variant of concern (VOC) by the WHO [[https://www.who.int/news/item/26-11-2021-classification-of-omicron-\(B.1.1.529\)-sars-cov-2-variant-of-concern](https://www.who.int/news/item/26-11-2021-classification-of-omicron-(B.1.1.529)-sars-cov-2-variant-of-concern)]. As compared with other VOCs, current evidence suggests an increased risk of reinfection with this variant.

It has been reported that distribution of mutants are found during SARS-CoV-2 replication in infected hosts (1–3), as was also previously described for other coronaviruses (4, 5) and in general for RNA viruses. This implies that a consensus sequence of an isolate determined for diagnostic purposes in reality hides a mixture of different variants present in different proportions within the same population (6).

Despite vaccination being highly effective in preventing severe COVID-19, vaccine breakthrough infections have been observed (7, 8). Little is known about the composition of the mutant spectra of SARS-CoV-2 that infect fully vaccinated individuals. This raises the question of whether a vaccine failure could be associated with an ensemble of variant genomes that can facilitate replication in

the face of an effective anti-SARS-CoV-2 immune response (9, 10). Here, we show that the virus replicating in vaccinated individuals who developed COVID-19 as a consequence of infection with the Alpha variant included signature mutations of Delta Plus, Iota, and Omicron SARS-CoV-2.

Results and Discussion

We studied 5 patients who had been fully vaccinated (2 doses) with BNT162b2 (Pfizer-BioNTech) and who mounted an effective antiviral response (>2000 AU/mL). They were subsequently infected with SARS-CoV-2 in April 2021 and developed COVID-19 clinical symptoms. Nasopharyngeal swabs were collected between April 6, 2021 and April 14, 2021, a time frame that corresponds to the fourth pandemic wave in Madrid, Spain, associated with the Alpha variant. RNA extracted from the diagnostic samples from these vaccinated and infected patients was used to amplify 6 overlapped amplicons of the genomic region of the spike (S) protein (covering nucleotides 21,424 to 23,666; residue numbering is according to the genomic nucleotide sequence of the Wuhan-Hu-1 isolate, NCBI reference NC_045512.2) that were analyzed by ultra-deep sequencing (UDS), with a cutoff value of 0.1%. Two deletions ($\Delta 69-70$ and $\Delta 144$) and 4 amino acid substitutions (N501Y, A570D, D614G, and P681H), characteristic of the Alpha variant, were dominant variations (termed “divergence” mutations) relative to the reference sequence (Wuhan-Hu-1 isolate) (Figure 1). Interestingly, in addition to these “divergence” mutations, we also found amino acid substitutions representative of the Delta Plus, Iota, and Omicron variants in the mutant spectra of the 5 patients

Conflict of interest: The authors have declared that no conflict of interest exists.

Copyright: © 2022, Martínez-González et al. This is an open access article published under the terms of the Creative Commons Attribution 4.0 International License.

Submitted: December 17, 2021; **Accepted:** March 1, 2022; **Published:** May 2, 2022.

Reference information: *J Clin Invest.* 2022;132(9):e157700.

<https://doi.org/10.1172/JCI157700>.