CORRESPONDENCE

SARS-CoV-2 Evolution and Immune Escape in Immunocompromised Patients

TO THE EDITOR: Mutations in the spike protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that confer escape from neutralizing antibodies can arise in immunocompromised patients with prolonged infection.[1,2] Such viral evasion is hypothesized to contribute to the emergence of global variants of concern.[3] In the absence of effective immune responses, selective pressures such as those from monoclonal antibody treatment may lead to the emergence of immunologically important mutations.

To understand selective pressures driving within-host SARS-CoV-2 evolution, we examined the relationship between such evolution and endogenous immune responses and exogenous antibody treatment in convenience samples obtained from five patients with B-cell deficiencies. (Details regarding each patient's clinical history are provided in the Supplementary Appendix, available with the full text of this letter at NEJM .org.) All the patients had SARS-CoV-2 infections lasting 42 to 302 days after a first positive test (day 0) (Fig. S1 and Table S1 in the Supplementary Appendix). The study was approved by the institutional review board at Emory University. Informed consent was obtained from the patients who donated whole blood samples for research (Patients 2, 4, and 5).

Patient 1 did not receive antibody treatment and was negative for neutralizing antibodies on day 37. Patients 2 and 3 were treated with the monoclonal antibody bamlanivimab on days 4 and 8, respectively. Their serum potently neutralized the reference pseudovirus (Wuhan-Hu-1) on day 33 (in Patient 2) and day 55 (in Patient 3) and retained elevated neutralizing-antibody titers through days 77 and 83, respectively (Fig. 1A). Patient 4 received convalescent plasma on days 0 and 104 and had undetectable neutralizing antibodies on days 82 and 101. Patient 5 received convalescent plasma on day 200 and had low neutralizing-antibody titers on day 204. Binding IgG titers to the spike protein reflected serum neutralization titers (Fig. S2). All but one patient (Patient 2) ultimately recovered. Patients 2, 4, and 5 provided peripheral-blood samples for immunophenotyping. All three of these patients had low lymphocyte counts and low-to-undetectable CD19+ B-cell frequencies (0.19% in Patient 2, 0.01% in Patient 4, and 0.01% in Patient 5) as compared with healthy controls and age-matched hospitalized patients with coronavirus disease 2019 (Covid-19) (Fig. S3). Patient 3 had clinically low levels of T and B cells. Thus, antibody responses against reference SARS-CoV-2 in Patients 2, 3, and 5 were probably due to exogenous treatments. SARS-CoV-2–specific effector T-cell responses were detectable in Patients 4 and 5, with CD8+ T cells secreting antiviral interferon-γ and tumor necrosis factor, but were detectable only at a background level in Patient 2 (Fig. 1B and 1C and Figs. S4, S5, and S6).

SARS-CoV-2 sequencing (Table S2 and Figs. S7 and S8) revealed spike protein evolution in Patients 2 and 3 (Fig. 1D and Fig. S9); both of these patients who had been treated with bamlanivimab were deficient in T and B cells. Consensus-level mutations and intrasample single-nucleotide variants were found in the spike receptor-binding domain (RBD) and N-terminal domain (NTD), regions that have been associated with immune escape.[4] In contrast, no RBD or NTD mutations were found in Patient 1, who did not receive antibodies, or in Patients 4 and 5, who received convalescent plasma and had intact T-cell responses to SARS-CoV-2.

To assess whether viruses obtained from Patients 1, 2, and 3 had been neutralized by autologous serum, we constructed infectious pseudoviruses expressing variant spikes (Fig. S10). Serum from Patients 1, 2, and 3 did not neutralize pseudoviruses with variant spikes, even though serum from Patients 2 and 3 neutralized the reference pseudovirus (Fig. S11). Thus, spike mutations in Patients 2 and 3 conferred neutralization resistance to bamlanivimab.

Our results underscore the potential importance of selective pressures such as the use of monoclonal antibodies — in combination with the lack of an effective endogenous immune re-

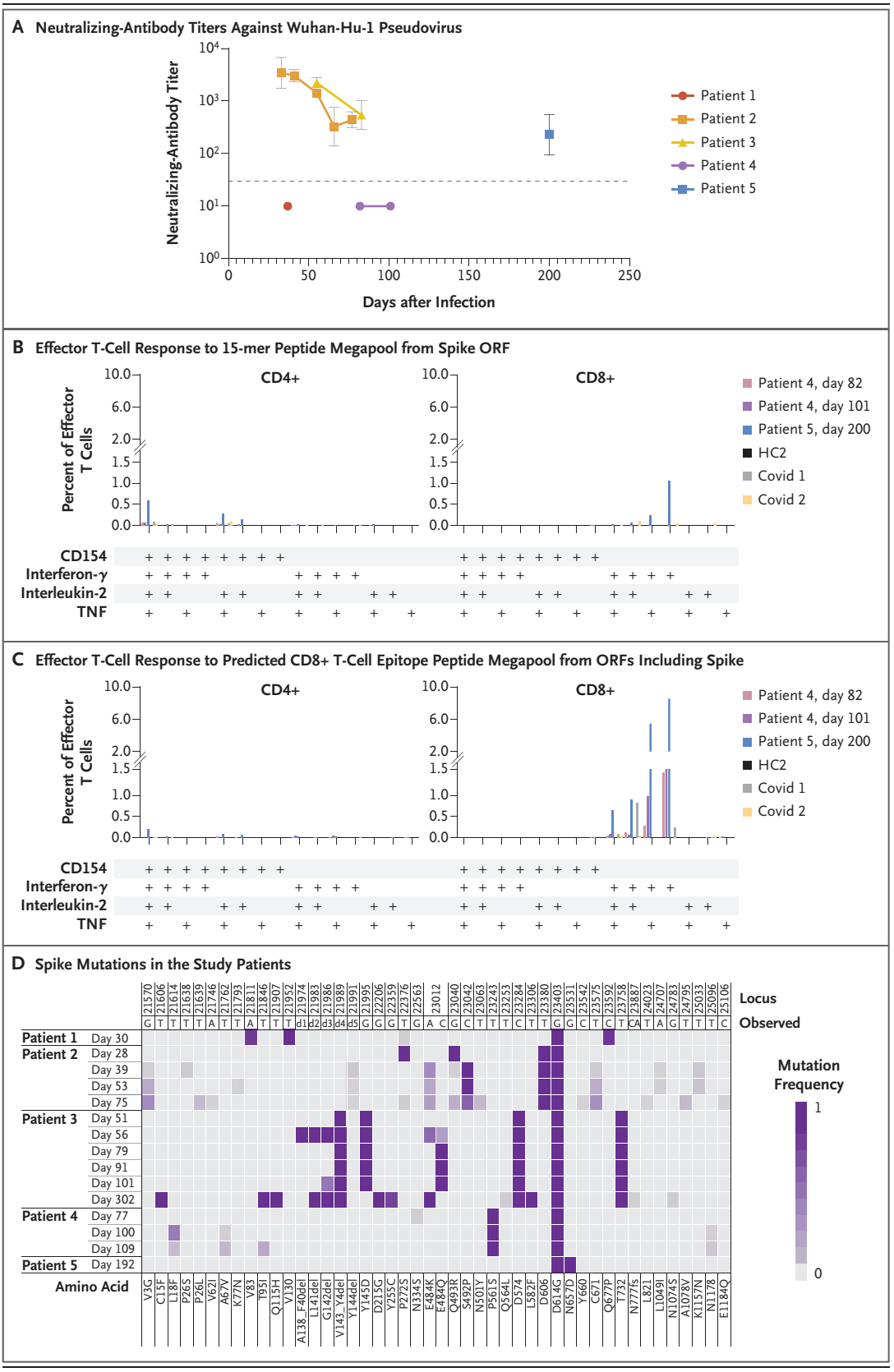
A Neutralizing-Antibody Titers Against Wuhan-Hu-1 Pseudovirus
Neutralizing-Antibody Titer
Days after Infection
Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
B Effector T-Cell Response to 15-mer Peptide Megapool from Spike ORF
CD4+
CD8+
Percent of Effector T Cells
CD154
Interferon-γ
Interleukin-2
TNF
Patient 4, day 82
Patient 4, day 101
Patient 5, day 200
HC2
Covid 1
Covid 2
C Effector T-Cell Response to Predicted CD8+ T-Cell Epitope Peptide Megapool from ORFs Including Spike
D Spike Mutations in the Study Patients
Locus
Observed
Mutation Frequency
Amino Acid

Figure 1 (facing page). Neutralizing-Antibody Titers, Effector T-Cell Responses, and Spike Mutations in Five Immunocompromised Patients.

Panel A shows neutralizing-antibody titers in patient serum against Wuhan-Hu-1, the reference SARS-CoV-2 pseudovirus, at various time points after infection. These titers represent the reciprocal serum dilution at which half-maximal pseudovirus neutralization was observed. Data show the geometric means of two to five independent experiments; I bars indicate standard deviations. The dotted line represents the lower limit of detection. Panels B and C show background-subtracted frequencies of CD4+ or CD8+ T cells expressing CD154, interferon-γ, tumor necrosis factor (TNF), or interleukin-2 as a percentage of non-naive (i.e., effector or memory) cells in response to stimulation of peripheral-blood mononuclear cells with a peptide megapool containing 15-mers from the spike open reading frame (ORF) and a peptide megapool containing predicted CD8+ T-cell epitopes from ORFs including spike, respectively. Frequencies were determined by flow cytometry in Patients 4 and 5, as well as in a healthy control donor (HC2) and two age-matched patients hospitalized with Covid-19 (Covid 1 and 2). Panel D shows mutations in the gene encoding the SARS-CoV-2 spike protein as compared with the Wuhan-Hu-1 strain, according to patient identifier and time point. Shading denotes mutation frequency. For each mutation, the observed variant nucleotide is listed above the plot and the amino acid mutation is listed below the plot.

sponse — in promoting the emergence of SARS-CoV-2 escape mutations. These findings highlight the need to better understand the ramifications of different therapies in immunocompromised patients. Our results also corroborate the findings of previous studies in which patients with B-cell deficiencies were found to elicit effector T cells,[5] an outcome that may signal an important role for T cells in controlling infection.

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Disclosure forms provided by the authors are available with the full text of this letter at NEJM.org.

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