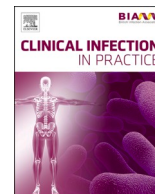




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Severe breakthrough COVID-19 with a heavily mutated variant in a multiple myeloma patient 10 weeks after vaccination

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ABSTRACT

Background: Patients with multiple myeloma have unpredictable responses to vaccination for COVID-19. Anti-spike antibody levels can determine which patients develop antibodies at levels similar to healthy controls, and are a known correlate of protection.

Case report: A multiple myeloma patient developed protective anti-spike antibodies after vaccination (608 IU/mL), but nonetheless developed severe breakthrough COVID-19 just 10 weeks following his second vaccination with mRNA-1273.

Results: Sequencing of the viral isolate revealed an extensively mutated variant with 10 spike protein mutations, including E484Q and N440K. Serology testing showed a dramatic decline in anti-spike antibodies immediately prior to virus exposure.

Conclusions: Multiple myeloma patients who do develop detectable antibody responses to vaccination may be at increased risk for breakthrough infections due to rapid decline in antibody levels. Viral variants with immune escape mutations such as N440K, also seen independently in the SARS-CoV-2 Omicron variant (B.1.1.529) and in viral passaging experiments, likely require a higher level of anti-spike antibodies to prevent severe COVID-19.

Introduction

SARS-CoV-2 has affected much of the world population, initially with no intervention to prevent spread other than social distancing. Administration of potent mRNA-based vaccines in the United States allowed vaccinated individuals to more safely resume social activities that exposed them to respiratory droplets. These vaccines are highly protective against severe disease and death from all strains, including the current variants of concern (Nasreen et al., 2021). Unfortunately, some immunocompromised individuals, including those with

hematologic malignancies, remain at risk for breakthrough infections due to inadequate immune responses to vaccination. Combined with the high transmissibility of SARS-CoV-2 and its tendency to cause worse outcomes among the immunocompromised (Lee et al., 2020), they remain at high risk for hospitalization and death.

Multiple myeloma (MM) is a bone marrow-based cancer characterized by rapidly proliferating plasma cells that produce monoclonal antibodies. These patients show impaired humoral immune responses with reduced uninvolved immunoglobulin levels. Myeloma therapies often further reduce the total B-cell and the more terminally-differentiated

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plasma cell populations since they indiscriminately target both malignant and nonmalignant cells. The disease and its treatments also often cause general bone marrow suppression as well as defective cell-mediated immunity. Infection is the leading cause of death among MM patients, at ~7–10-fold higher risk compared to the general population (Blimark et al., 2015). MM patients have reduced vaccine-induced protection from viral diseases, and develop lower levels of anti-spike antibodies following vaccination for COVID-19 (Robertson et al., 2000; Stampfer et al., 2021).

We describe a case of a patient with MM who responded normally to vaccination based on his anti-spike antibody level following mRNA-1273 administration, but who still developed severe COVID-19 infection to which he fully recovered following a high-grade exposure to a highly mutated SARS-CoV-2 variant.

Methods

Quantitative Anti-Spike IgG ELISA

The semiquantitative spike IgG ELISA assay was described previously and outputs data in international units (IU)/mL, with 1000 IU/mL matching the 20/136 NIBSC WHO convalescent plasma standard (Stampfer et al., 2021).

Sequencing

Samples were processed and sequenced as described previously (Zhang et al., 2021; Zhang et al., 2020). Results were uploaded to GISAID (accession number: EPI_ISL_4062607).

Case description

A 61-year-old Hispanic male with kappa light chain MM in complete remission since September 2020 was on maintenance therapy with ixazomib, lenalidomide and oral methylprednisolone. Shortly before he received his COVID-19 vaccination, myeloma labs on 1/26/21 showed no detectable monoclonal protein with his IgG at 776 mg/dL (reference range 540–1822 mg/dL), IgA 131 mg/dL (101–645 mg/dL), IgM 47 mg/dL (22–240 mg/dL), serum free light chain kappa and lambda levels of 15.7 mg/L (3.3–19.4 mg/L) and 22.7 mg/L (5.7–26.3 mg/L), respectively, and an absolute lymphocyte count of $1.52 \times 10^3/\mu\text{L}$ ($1-5 \times 10^3/\mu\text{L}$). He had no evidence of pre-existing immunity to COVID-19, with a baseline anti-spike IgG of 5.74 IU/mL well below the assay background of 50 IU/mL (Stampfer et al., 2021).

He received mRNA-1273 vaccine doses on 1/30/21 and 2/24. Anti-spike IgG levels were 34.6 IU/mL (18 days after dose 1) and 608 IU/mL (21 days after dose 2; Fig. 1A). This was thought to confer significant protection, as the second value was 37th percentile when compared to similar specimens from vaccinated healthy controls in our recently published study (Stampfer et al., 2021). He did experience significant decay of spike-specific IgG, declining to 126 IU/mL 8 weeks after his second dose, consistent with a half-life of 16.3 days (calculated by exponential decay).

He left the United States and flew to Mexico on 4/25. On 4/28, he met with his unvaccinated coworkers (Pt2 and Pt3) without masks in an indoor setting and in close proximity in Mazatlán, in the Mexican state of Sinaloa. On the following day, he met with Pt2 again. Shortly after returning home on 5/1, he developed pharyngitis, myalgias, rhinitis, and severe coughing resulting in a subconjunctival hemorrhage. He subsequently developed diaphoresis and fever to 101°F, and his PCR test detected COVID-19 on 5/8, with cycle thresholds of 20.8 (N-gene) and 21.8 (E-gene) corresponding to a VL of ~400,000. At that time, he met

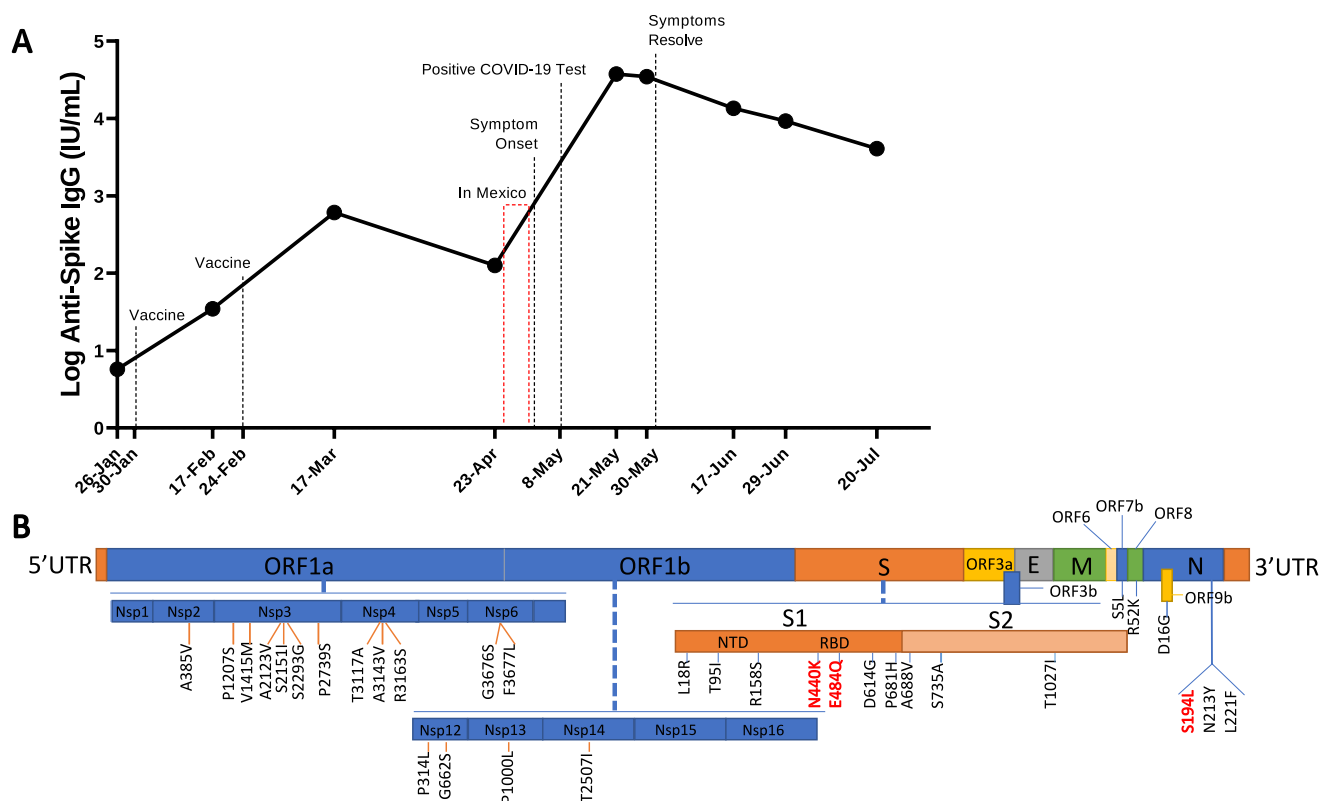


Fig. 1. Anti-Spike Antibodies and Mutations in a Breakthrough Infection. **A:** Anti-spike IgG levels measured at serial intervals pre-vaccination, post-vaccination and post-infection. Key events are indicated with dashed lines; dates correspond to serum samples. **B:** Mutations noted on the patient's SARS-CoV-2 isolate. Notable mutations for pathogenicity and immune escape are bolded and in red. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

criteria for severe COVID-19 with an oxygen saturation of 92% but declined hospitalization and returned home. Symptoms persisted for another 10 days before beginning to improve, and he recovered fully during the next month. A follow-up spike antibody level drawn on 5/21, 13 days after the positive PCR for COVID-19 and 23 days after the likely exposure on 4/28, increased over 250-fold to 37,400 IU/mL. It remained elevated 7 days later at 34,700 IU/mL before declining precipitously, reaching 4070 IU/mL on 7/20 with a calculated half-life of 16.5 days, similar to his pre-infection antibody decay. Incidentally, his family all had close contact with him following his return to the United States on 5/1, but all tested negative for COVID-19 on 5/8 and 5/12, including an unvaccinated daughter.

His clinical course was significantly milder than his unvaccinated coworkers Pt2 and Pt3, who likely contracted COVID-19 at the same time. Both were from Culiacán in Sinaloa, Mexico. Both had a history of asthma but were otherwise healthy, and both were hospitalized with COVID-19 shortly on 5/8, 7 days after the onset of his symptoms. Pt2 required intubation, with a two-week hospitalization complicated by new onset heart failure. Pt3 was hospitalized for 5 days and required supplemental oxygen. Both patients recovered.

Our patient's COVID-19 isolate was sequenced and found to have 45 mutations relative to the original SARS-CoV-2 isolates from early 2020 (Fig. 1B), consistent with an extensively mutated form of the B.1.628 variant (24 canonical mutations) (Latif et al., 2021). This included three mutations in the nucleocapsid and 10 in the spike gene. Both mutations in the receptor-binding domain (RBD) of spike, N440K and E484Q, were in only our patient's strain. At the time, 5 similar extensively mutated variants had been reported in the GISAID database, all from the state of Sinaloa in Mexico, consistent with an exposure during his time in Mazatlán on 4/27–5/1. His exposure most likely occurred on 4/28 given that the two colleagues he saw in Sinaloa that day had a disease course with symptoms and timing that paralleled his own.

Discussion

This case demonstrates the role and limitations of COVID-19 vaccination for MM patients. Following vaccination, our patient's antibody level of 608 IU/mL corresponded to 37th percentile compared to vaccinated healthy controls, which suggested some clinical protection from. However, he was still immunocompromised from both his immunosuppressive regimen to treat his MM as well as from the disease itself, despite being in complete remission. His antibody level fell rapidly to 126 IU/mL just 6 weeks later, indicative of a serum half-life of just 16.3 days following vaccination, and likely contributed to his breakthrough infection. Concerningly, his calculated half-life after infection was similar at 16.5 days, suggesting that he may again become susceptible to reinfection in spite of transiently high antibody levels. Vaccinated healthy individuals have much longer half-lives, averaging 52 days when measured against receptor-binding domain antibodies (Doria-Rose et al., 2021). His short half-lives are more consistent with the 11–30 day half-life observed with exogenous antibody infusions (Ovacik and Lin, 2018), suggestive of limited ongoing anti-spike antibody production in this patient.

Without defined correlates of protection, it is difficult to quantify the clinical impact of COVID-19 vaccination in this patient. It is noteworthy that his two contacts were both hospitalized from severe COVID-19, one with critical illness, and suggests that all three individuals experienced a high inoculum SARS-CoV-2 exposure. His disease was milder than that of his two unvaccinated contacts in spite of their lack of known immunocompromising conditions, suggesting that the vaccine provided partial protection for this patient and may have prevented critical illness and even death. Moreover, family members with close patient contact remained uninfected, suggesting that the pre-existing immunity from his prior vaccination may have helped prevent further transmission to these highly exposed individuals.

Further analysis of his COVID-19 type showed his isolate to be

extensively mutated (Fig. 1B), including 10 mutations in spike; 3 localized to the N-terminal domain (NTD), a target of neutralizing antibodies. The E484Q mutation in the RBD, not present in the original B.1.628 isolate, is also present in the kappa variant of interest and thought to confer immune evasion similar to E484K, which alone reduced neutralizing titers by a median of 2.8-fold among recipients of mRNA vaccines for COVID-19 (Wang et al., 2021). N440K—also absent from the original B.1.628—has been observed in viral passaging experiments in the presence of convalescent plasma and provides additional immune escape *in vitro* (Wang et al., 2021). Notably, N440K is also present in the SARS-CoV-2 Omicron variant (B.1.1.529) (O'Toole et al., 2021a, 2021b), and has been shown to be associated with breakthrough infections *in vivo* (Rani et al., 2021). Of the three nucleocapsid mutations noted in our isolate, S194L has been best characterized, and is associated with poorer clinical outcomes (Nagy et al., 2021). Our patient's isolate contained 45 amino acid changes throughout the SARS-CoV-2 genome, nearly as many as in Omicron, consistent with extensive viral evolution of both variants.

We describe a case of COVID-19 breakthrough infection with several unique features. It occurred in an immunologically complex multiple myeloma patient who developed more severe symptoms than would be expected for a breakthrough infection, possibly due to a high viral inoculum. The cause was an extensively mutated B.1.628 SARS-CoV-2 variant, featuring N440K and E484Q spike mutations. As SARS-CoV-2 variants continue to diverge and evolve mutations that escape pre-existing immunity, such infections may become commonplace among immunocompromised individuals. They may ultimately benefit from revaccination, prophylactic antibody infusions and increased, consistent social distancing directed at reducing SARS-CoV-2 exposure levels. Further data on correlates of protection is required to be able to determine whether spike IgG levels can be used for guidance regarding these specific COVID-19 protective approaches.

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Patient consent statement

Procedures followed were in accordance with the ethical standards of Helsinki Declaration of the World Medical Association. Written consent was obtained from patients and controls.

CRedit authorship contribution statement

Samuel D. Stampfer: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization, Supervision, Funding acquisition. **Marissa-Skye Goldwater:** Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Sean Bujarski:** Methodology, Software, Validation, Formal analysis, Data curation, Visualization. **Bernard Regidor:** Investigation, Data curation. **Wenjuan Zhang:** Methodology, Software, Validation, Formal analysis, Investigation, Data curation. **Aaron J. Feinstein:** Validation, Investigation. **Regina Swift:** Project administration. **Shahrooz Eshaghian:** Investigation. **Eric Vail:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – review & editing, Supervision, Project administration, Funding acquisition. **James R. Berenson:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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