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US Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Epsilon Variant: Highly Transmissible but With an Adjusted Muted Host T-Cell Response

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Background. The multiple mutations comprising the epsilon variant demonstrate the independent convergent evolution of severe acute respiratory syndrome coronavirus (SARS-CoV-2), with its spike protein mutation L452R present in the delta (L452R), kappa (L452R), and lambda (L452Q) variants.

Methods. Coronavirus disease 2019 (COVID-19) variants were detected in 1017 patients using whole-genome sequencing and were assessed for outcome and severity. The mechanistic effects of the epsilon versus non-epsilon variants were investigated using a multiomic approach including cellular response assays and paired cell and host transcriptomic and proteomic profiling.

Results. We found that patients carrying the epsilon variant had increased mortality risk but not increased hospitalizations ($P < .02$). Cells infected with live epsilon compared with non-epsilon virus displayed increased sensitivity to neutralization antibodies in all patients but a slightly protective response in vaccinated individuals ($P < .001$). That the epsilon SARS-CoV-2 variant is more infectious but less virulent is supported mechanistically in the down-regulation of viral processing pathways seen by multiomic analyses. Importantly, this paired transcriptomics and proteomic profiling of host cellular response to live virus revealed an altered leukocyte response and metabolic messenger RNA processing with the epsilon variant. To ascertain host response to SARS-CoV-2 infection, primary COVID-19–positive nasopharyngeal samples were transcriptomically profiled and revealed a differential innate immune response ($P < .001$) and an adjusted T-cell response in patients carrying the epsilon variant ($P < .002$). In fact, patients infected with SARS-CoV-2 and those vaccinated with the BNT162b2 vaccine have comparable $CD4^+/CD8^+$ T-cell immune responses to the epsilon variant ($P < .05$).

Conclusions. While the epsilon variant is more infectious, by altering viral processing, we showed that patients with COVID-19 have adapted their innate immune response to this fitter variant. A protective T-cell response molecular signature is generated by this more transmissible variant in both vaccinated and unvaccinated patients.

Keywords. COVID; variant; T-cell response; transcriptomics; proteomics.

The coronavirus disease 2019 (COVID-19) global pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to evolve, with continuous emergency of novel variants. These include variants seen in the United Kingdom (alpha/20I/501Y.V1/B.1.1.7), South Africa (beta/20H/501Y.V2/B.1.351), and Brazil (gamma/P.1/20J/501Y.V3/B.1.1.248), which remain of concern owing to increased infectivity and virulence [1–5], with the latest variant of concern arising from the convergent evolution of the delta/20A/B.1.617.2/S:478K variant from India [6].

In January 2021, our group discovered a new SARS-CoV-2 variant, epsilon, the first reported US variant [7]. The epsilon variant, also now known as B.1.429/B.1.427/S:452R, is defined by the following mutations: ORF1b, D1183Y; S protein, S13I; W152C;

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and L452R. In particular, mutations in the SARS-CoV-2 spike protein raise concern regarding this variant's transmissibility, pathogenicity, and effect on vaccine efficacy, increasing the urgency of understanding its clinical relevance. The epsilon variant shows increased transmissibility [8] and thus was elevated to a variant of interest by the Centers for Disease Control and Prevention [9]. The delta variant demonstrates convergent evolution of SARS-CoV-2 by independently acquiring the defining spike protein mutation seen in the epsilon variant L452R and a similar mutation at P681 seen in the alpha variant. With the exception of the D614G mutation, L452R and P681 are the only shared mutations seen in other variants and the delta. Other emerging variants of interest, lambda (South America; 452R) and kappa (India; 452Q), also share the 452R mutation [10], further demonstrating the ability for SARS-CoV-2 to acquire similar mutations independently throughout the world.

The 452R mutation, seen in the delta, epsilon, lambda, and kappa variants [11], is in the angiotensin-converting enzyme 2 receptor binding domain of the spike protein and has been found to alter neutralization antibody (NAb) responses in pseudoviral assays [12]. This finding, along with evidence that the epsilon variant is more transmissible [13], is important because this functionally plastic region can also [12] evolve to have decreased virulency. Hence, we sought to systematically elucidate the pathogenic and molecular mechanisms caused by the epsilon variant at a cellular level in Vero cells and at a host level in patient samples.

The current study investigated the impact of the epsilon variant on clinical outcome, virulency, vaccine efficacy, and host T-cell response. Because some COVID-19 vaccines are based on the ancestral spike protein amino acid sequence, a global concern of vaccine evasiveness arises as emerging SARS-CoV-2 variants have spike protein mutations. To address this, nonvariant and epsilon variant serum were used to test whether this variant is as sensitive to NAbs [12]. While the correlates of protection in convalescent individuals and vaccines are unknown, it is assumed that both a broad humoral and a cell-mediated immunological response are most likely necessary to fully protect against COVID-19. NAbs almost certainly serve as the first line of defense against infection, but the $CD4^+/CD8^+$ T-cell responses are also important for prevention of further disease progression. Early reports of the epsilon variant identified a broad $CD8^+$ T-cell response in virtually all individuals having detectable responses to several viral epitopes [14, 15]. While these assays help show viral virulence, they are limited in (1) the type of virus used (eg, pseudotyped viral particles), (2) the narrow cell response captured, and (3) the lack of data on host adaptive immunity.

Viruses such as SARS-CoV-2, besides having innate immunity, induce both humoral and cellular adaptive immune responses, triggering different host defense mechanisms to fight an acute infection. The current study comprehensively investigated innate and humoral host responses, along with transcriptomic and proteomic analyses of the epsilon variant to properly assess its effect in vaccinated and unvaccinated individuals. Its findings study demonstrate that while SARS-CoV-2 emerging variants can increase their viral infectivity, the evolving arms race also shows that the host immune system is altering its immunogenicity to increase its protective response to these variants, resulting in an adjusted T-cell response. These findings have direct public health implications, with the continuous global assessment of the convergent evolution of SARS-CoV-2 variants and vaccine efficacy.

METHODS

Diagnostics and Sample Preparation and Next-Generation Sequencing Variant Analyses

All experiments were conducted following institutional review board (IRB) protocols approved by the Cedars-Sinai Medical Center (CSMC) Office of Research Compliance and Quality Improvement (IRB no. 629_MOD6887). Patients were first assessed with reverse-transcription quantitative polymerase chain reaction (Accelerate Technologies, Singapore) for SARS-CoV-2 viral RNA and diagnostically COVID-19 positive with amplification of the targeted region crossing the threshold before 30 cycles using previously established protocols. In total, 1017 COVID-19–positive samples were used for parallel next-generation sequencing analysis. All CSMC-hospitalized patients with a positive polymerase chain reaction test result during this time period were included in the study. For information on diagnostics, sample preparation, next-generation sequencing analysis, and cell culture see the Supplementary Methods and sample usage in Supplementary Figure 1.

Propagation of SARS-CoV-2 Virus and Infectivity Assay

Using standard cell culturing methods (Supplementary Methods), all live viral culture experiments were performed in a certified biosafety level 3 laboratory. Replication competent SARS-CoV-2 control isolate (D614G) and SARS-CoV-2 epsilon variant was isolated from nasopharyngeal positive swab samples using Vero E6 cells (7×10^6 cells) grown in complete growth medium. In brief, a viral inoculum (multiplicity of infection, 0.1) was prepared for 3 identified epsilon strains of SARS-CoV-2 in serum-free culture medium and incubated for 4–6 hours. Progression of the infection was verified by the appearance of viral plaques. Cell culture supernatants were harvested at the 96-hour time point, and cell debris was removed by centrifugation. Viral titers were measured using a standard viral plaque assay, and viral growth kinetics were observed using viral genome replication assay (Supplementary Methods).

Live Virus Neutralization Assay

Plasma samples were obtained from 10 individuals vaccinated with the Pfizer BNT16b2 messenger RNA (mRNA) vaccine

(collected 22 March 2021, 4 weeks after vaccination). Convalescent plasma and postvaccine plasma for both epsilon and non-epsilon samples were 2-fold serially diluted using serum-free medium. All samples were tested in triplicate using methods outlined in the Supplementary Materials.

T-Cell Assay

SARS-CoV-2–specific T-cell levels were measured by means of cytokine flow cytometry. Briefly, fresh whole blood was incubated overnight with overlapping peptide mixtures of 315 peptides spanning the sequence of SARS-CoV-2 non-epsilon or epsilon variant spike glycoprotein (JPT) together with brefeldin A and anti-CD28/CD49d (BD Biosciences). The percentage of $CD4^+$ cells expressing interleukin 2 and tumor necrosis factor α and the percentage of S-peptide–stimulated $CD8^+$ cells expressing interferon γ and tumor necrosis factor α were calculated and defined as CoV2-Th and CoV2-Tc, respectively. Negative and positive controls included cells not incubated with S-peptides and those stimulated with phytohemagglutinin.

Cellular and Host Response Transcriptomic Profiling

For cellular-response transcriptomic profiling, RNA (ribonucleic acid) extraction from cells of NAb assays was performed (Machery Nagel; no. 740984.250), and for RNA sequencing (RNAseq) analysis of cellular response, RNAseq libraries from cells (NAb) and from nasopharyngeal patient samples (10 epsilon and 10 non-epsilon) were prepared using an Illumina Truseq stranded mRNA kit. For host response transcriptomics, low-input RNAseq was conducted on total nucleic acid extracted from nasopharyngeal swab samples from 30 patients with COVID-19 (15 non-epsilon and 15 epsilon), prepared using a Qiaseq Ultra Low Input library preparation kit (Qiagen). All processing and data analysis were performed using standardized and previously reported pipelines (Supplementary Methods).

Whole-Cell Lysate Proteomics

Frozen cell pellets were lysed and protein solubilized and denatured with 1-mol/L ammonium bicarbonate in 8-mol/L urea, and subsequent ultrasonicated with 5 successive 10-second pulses. Then 35 μg of protein, based on Pierce BCA (Thermo Fisher Scientific), underwent cysteine reduction with 10-mmol/L dithiothreitol; alkylation with iodoacetamide (100 mmol/L); trypsin digestion at a ratio of 1 μg of trypsin to 35 μg of total protein for 16 hours at 37°C; and desalting on a 96-well HLB microelution plate (Waters) after acidification. Data-independent acquisition-mass spectrometry and downstream data analysis were performed according to established protocols (Supplementary Methods).

RESULTS

Viral Kinetics of the Epsilon Variant

We investigated the genomic characteristics and clinical outcomes associated with epsilon infection in patients admitted to CSMC (Supplementary Figure 1). Viral load differences were assessed between patients infected with this variant and those infected with previously circulating lineages, and findings were consistent with a previous report [13]. Viral genomes were sequenced from nasopharyngeal swab samples collected from patients with SARS-CoV-2 infection from October 2020 to June 2021. The study dates were selected because the epsilon variant was first detected in October 2020 and became the dominant variant in Southern California by January 2021, dates coinciding with a surge in hospitalizations (Figure 1). The epsilon variant peaked in this population in February (72%) and became severely reduced by March 2021 (48%) and minimally detected at a time coinciding with the emergence of the delta variant by April to June 2021 (Supplementary Figure 2). To explore differences in the clinical severity associated with the epsilon variant, we examined clinical information from this patient cohort. Although no significant difference in hospitalization was detected between patients carrying the epsilon versus the non-epsilon variant, the mortality rate was increased in epsilon-variant patients ($P < .02$).

To examine the replication kinetics of the SARS-CoV-2 non-epsilon and epsilon variant, we infected human Vero E6 cells, a cell line widely used to propagate the novel coronavirus [16], with each of the viral isolates at 24-, 48-, 72-, and 96-hour time points. During early stages of infection (24 hours), the epsilon variant had a 2-fold increase in infectious progeny compared with the non-epsilon variant. At 48 hours after infection, the epsilon growth curve showed a 3-fold increase relative to the non-epsilon variant, but by 72–96 hours after infection both variants reached a growth plateau (Figure 2*A*). Consistent with this, plaque formation was more prominent for the non-epsilon variant in the early stages of infection, despite both viral variants transitioning to large plaque formation by 96 hours after infection (data not shown).

Cellular Response to Live Viral Infection With the Epsilon Variant

The NAb assay was performed on both convalescent plasma ($n = 10$) from symptomatic patients infected with SARS-CoV-2 in May 2021. Both non-epsilon and epsilon variants were found to be significantly neutralized ($P < .001$) by both the convalescent plasma and the plasma from vaccinated participants, compared with the no-treatment growth control (Figure 2*B* and 2*C*). A comparison of effective neutralization showed decreased neutralization for the epsilon variant compared with the non-epsilon variant, with decreases of approximately 4-fold for convalescent plasma and 2-fold for plasma from vaccinated individuals (Figure 2*D* and 2*E*).

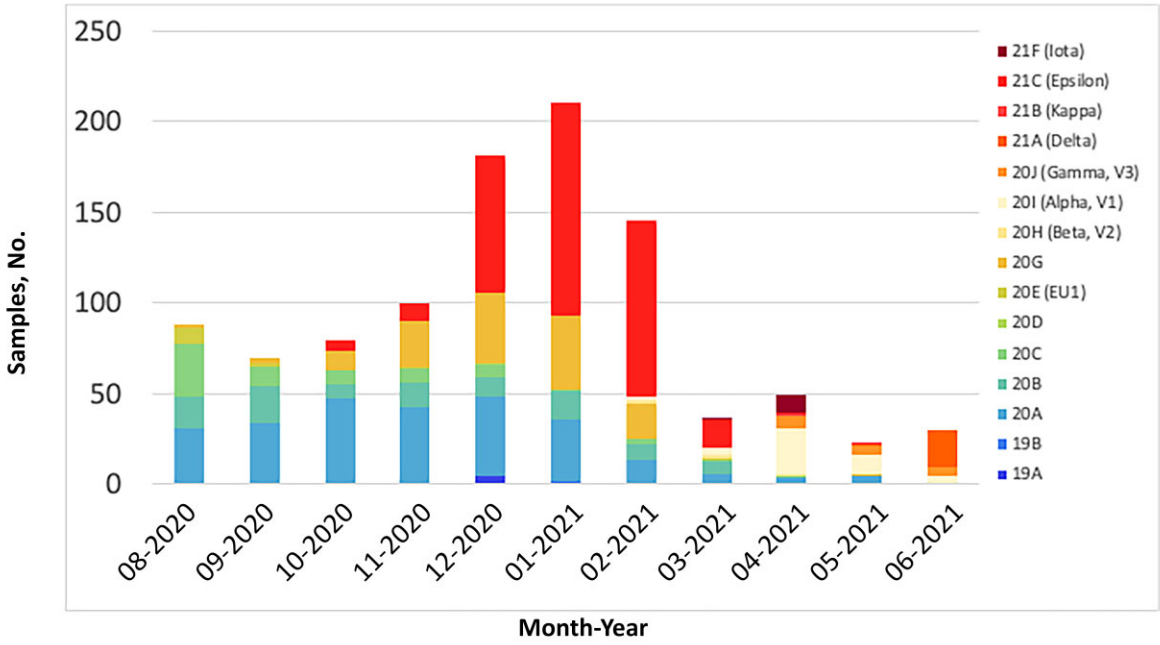

Figure 1. Diagrammatic representation of circulating severe acute respiratory syndrome coronavirus 2 variant frequencies from the Cedars-Sinai Medical Center catchment cohort (n = 1017) These samples were collected from patients with coronavirus disease 2019, from August 2020 to June 2021.

Muted Response to Cell Invasion Seen With Multiomic Functional Profiling of Epsilon Variant

Given that the epsilon variant demonstrated an altered cellular response on live viral infection, we sought to characterize molecularly the host response accounting for these changes, using paired transcriptomic and proteomic analyses at 24 hours after infection to compare non-epsilon and epsilon variant SARS-CoV-2 virus (Figure 3*A*). We found 300 differentially expressed genes (DEGs) and differentially expressed proteins (DEPs) in epsilon variant–infected cells compared with the non-epsilon variant (Supplementary Table 1). Within the total 2372 genes and proteins shared across RNA and protein, fold changes had a 58.5% consensus for the same directionality. Among those, 300 overlapping DEG/DEPs shared 72.7% fold changes consensus in fold change directionality (Supplementary Table 1). Functional annotation analysis of these 300 overlapping DEG/DEPs revealed down-regulation and corresponding reduction in the quantities of mRNA and proteins in gene ontology pathways associated with (1) viral mRNA processing (viral transcription, viral process, viral mRNA translation), (2) host mRNA processing, (3) protein expression (RNA transport and binding, mRNA stability and splicing, ribosome, cytoplasmic translation, protein folding and localization), and (4) heat shock protein response (Figure 3*B* and 3*C*).

These pathways are implicated in the reduced cellular stress response of cells infected with epsilon versus non-epsilon variant. Conversely, gene ontology enrichment analysis showed an up-regulation in epsilon variant–treated cells in pathways involved in (1) leukocyte-mediated immunity (Figure 3*B* and 3*D*), (2) cell–extracellular space interactions, and (3) several metabolic pathways in cell cytoplasm (Figure 3*B*). While overall mRNA processing and protein expression machinery processes were aligned across both transcriptomes and proteomes, several notable central metabolism processes—including citric acid cycle, glycolysis, and the hexose metabolic process—were up-regulated in the RNAseq analysis yet down-regulated on the protein expression level, which might be a manifestation of a delayed metabolic response at that level.

Epsilon Variant Alteration of Host Innate Immune Response With Intact T-Cell Response

To investigate direct host immune response, primary nasopharyngeal swab samples from patients with non-epsilon or epsilon variant COVID-19 and high viral loads were profiled by RNAseq. DEG analysis of the epsilon versus non-epsilon samples demonstrated 87 up-regulated and 22 down-regulated genes (Supplementary Table 2). From gene ontology enrichment analysis, these DEGs overlapped with altered cellular response pathways seen in joint transcriptomic/proteomic analysis in Vero E6 cells. These included mRNA processing, splicing, integrin binding, leukocyte infiltration, secretory vesicles, and granule transport (Figure 4*A* and 4*B*).

When comparing individuals infected with the epsilon variant, we observed that up-regulated genes were functionally enriched in processes involved in antigen processing and

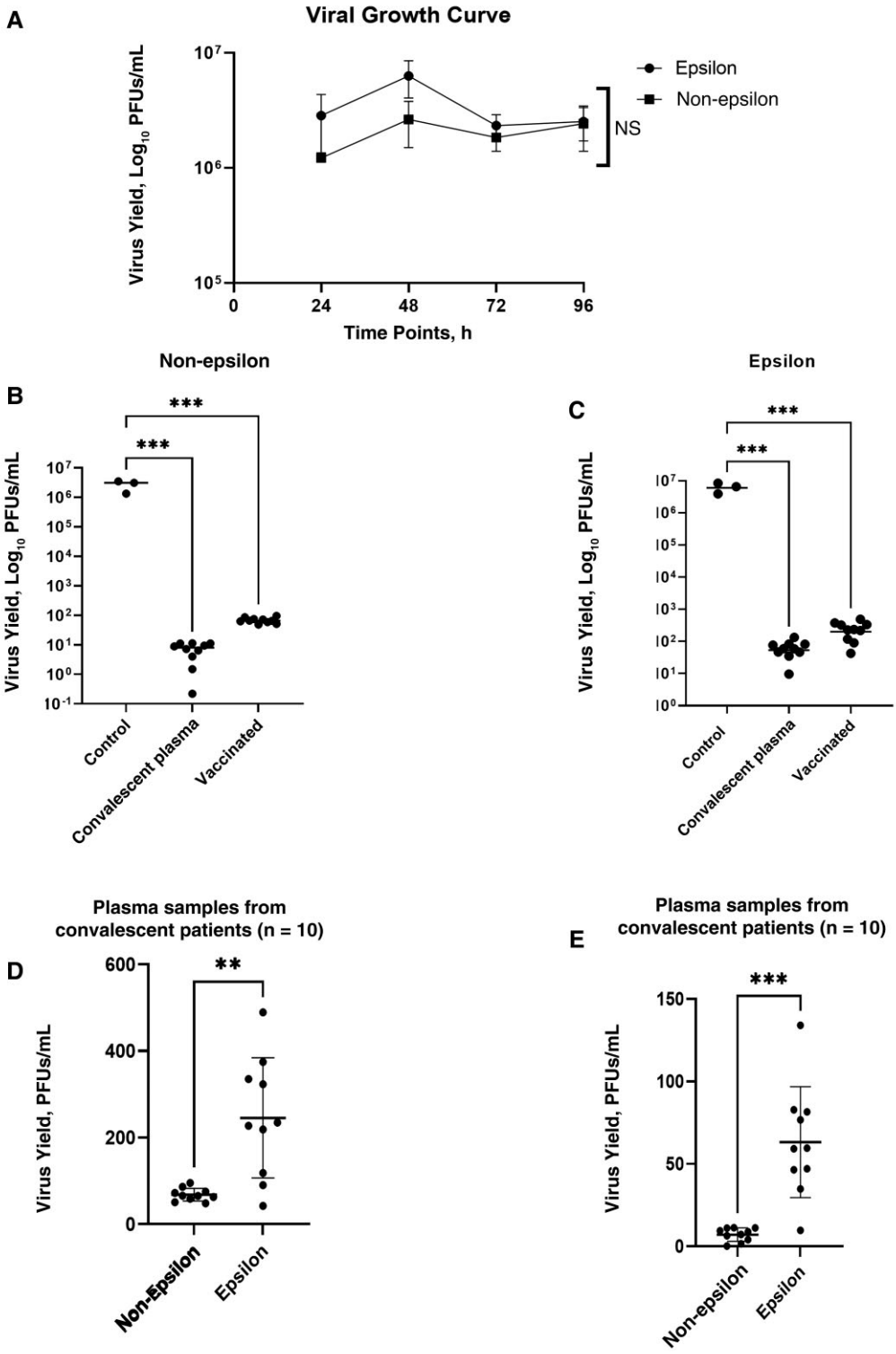

Figure 2. Comparison of severe acute respiratory syndrome coronavirus 2 non-epsilon variant and epsilon variant replication kinetics and neutralization effect in plasma samples from convalescent patients and from vaccinated individuals. *A*, Growth curves illustrating the relative concentration (in plaque-forming units [PFUs] per milliliter) of infectious viral progeny when grown in Vero E6 cells for 24, 48, at 72 hours after infection with non-epsilon (control, D614G) and epsilon (L452R) variants. Abbreviation: NS, not significant. *B, C,* Total neutralization effect for both convalescent plasma samples (n = 10) and plasma samples from vaccinated participants (n = 10) for both control variant and epsilon variant compared with no-treatment controls (n = 3). *D, E,* Viral loads for both the control variant and epsilon variant in plasma samples from vaccinated participants (*D*) and from convalescent patients (*E*). $^{**}P < .01$; $^{***}P < .001$.

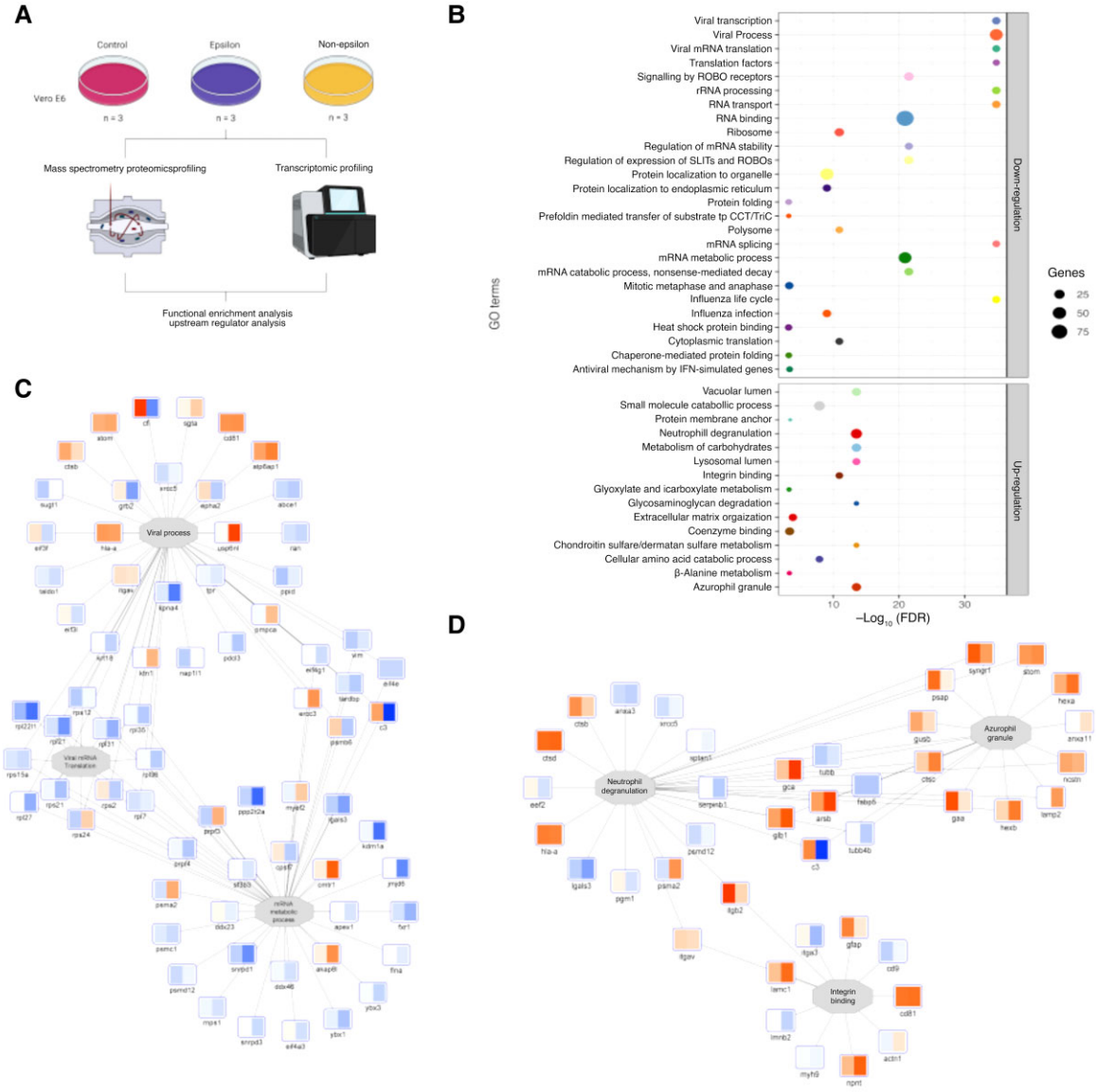

Figure 3. Multiomic profiling of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) epsilon variant host response in Vero E6 cells, compared with control non-epsilon variant. Schematic illustration shows the experimental design of the multiomic analyses. *A,* Differential expression between epsilon variant and wild-type variants demonstrating significantly enriched gene ontology terms in down-regulated (*top panel*) and up-regulated (*bottom panel*) genes and proteins. *B,* Down-regulated pathways showing selected gene ontology (GO) terms for networks of genes (*left box*) and proteins (*right box*) with overlaid $\log_2$ fold change value heat maps. Abbreviations: CCT, Chaperonin Containing TCP-1; FDR, false discovery rate; IFN, interferon; mRNA, messenger RNA; ROBO, roundabout; rRNA, ribosomal RNA; SLIT, slit guidance ligand; TriC, T-complex protein Ring Complex. *C,* $\log_2$ fold changes, with blue indicative of a decrease, and orange of an increase in epsilon compared with wild-type variants. *D,* Selected up-regulated pathways of genes and proteins shown in $\log_2$ fold change heat maps.

presentation, pathways associated with leukocyte infiltration, T-cell differentiation, and the T-cell–mediated immune response. The up-regulated processes in the epsilon variant may affect and explain infectivity during its emergence. Unsupervised clustering of the non-epsilon versus epsilon variant transcriptomes revealed alteration in immune innate host response signaling pathways ($P < .001$; Figure 4C). To address concerns of host response altered efficacy, we looked specifically at the T-cell signaling pathway response and observed some global changes to T-cell response pathway gene expression ($P < .002$; Figure 4D).

We next determined whether the epsilon variant has the capacity to evade T-cell recognition and response pathways generated by SARS-CoV-2 infection and vaccination. The T-cell response to the variant epsilon spike protein was determined in 15 infected patients and 18 vaccinated individuals 1 month after the second dose of Pfizer BNT162b2 mRNA vaccine. As shown in Figure 5A, $CD4^+$ T cells from infected patients and

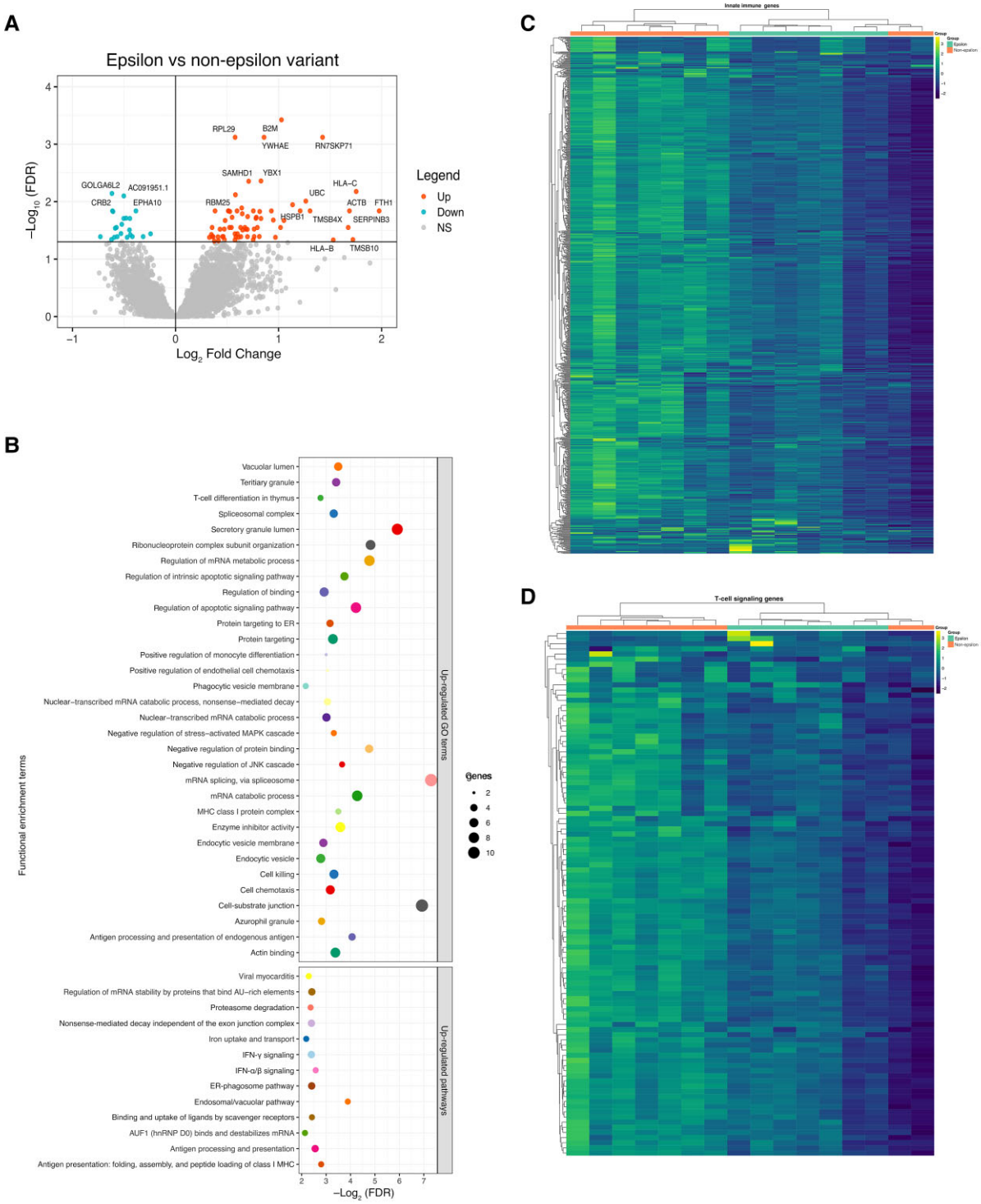

Figure 4. Host transcriptomic profiling and differential gene expression analysis of coronavirus disease 2019 (COVID-19)–positive patients, comparing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) epsilon variant and with control non-epsilon variant. *A, B,* Differential gene expression analysis between epsilon versus non-epsilon variant infected individuals (*A*) demonstrating Volcano plot of significantly up-regulated and down-regulated genes in epsilon variant (*B*) demonstrating gene ontology (GO) cellular component, biological process and molecular function enriched in up-regulated genes (*top panel*) and up-regulated biomolecular pathways (REACTOME, KEGG, Wiki pathways [*bottom panel*]). Abbreviations: AU, Adenylate-uridylate; AUF1, AU-rich element RNA-binding protein 1; ER, endoplasmic reticulum; FDR, false discovery rate; hnRNP, heterogeneous nuclear ribonucleoproteins; IFN, interferon; JNK, Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MHC, major histocompatibility complex; mRNA, messenger RNA; NS, not significant. *C,* Unsupervised clustering heat map demonstrates that epsilon share the most similar profiles in their differentially expressed genes when compared with non-epsilon variant in pathways involved in innate immune response. *D,* Pathway enrichment analysis between patients containing epsilon and non-epsilon variants.

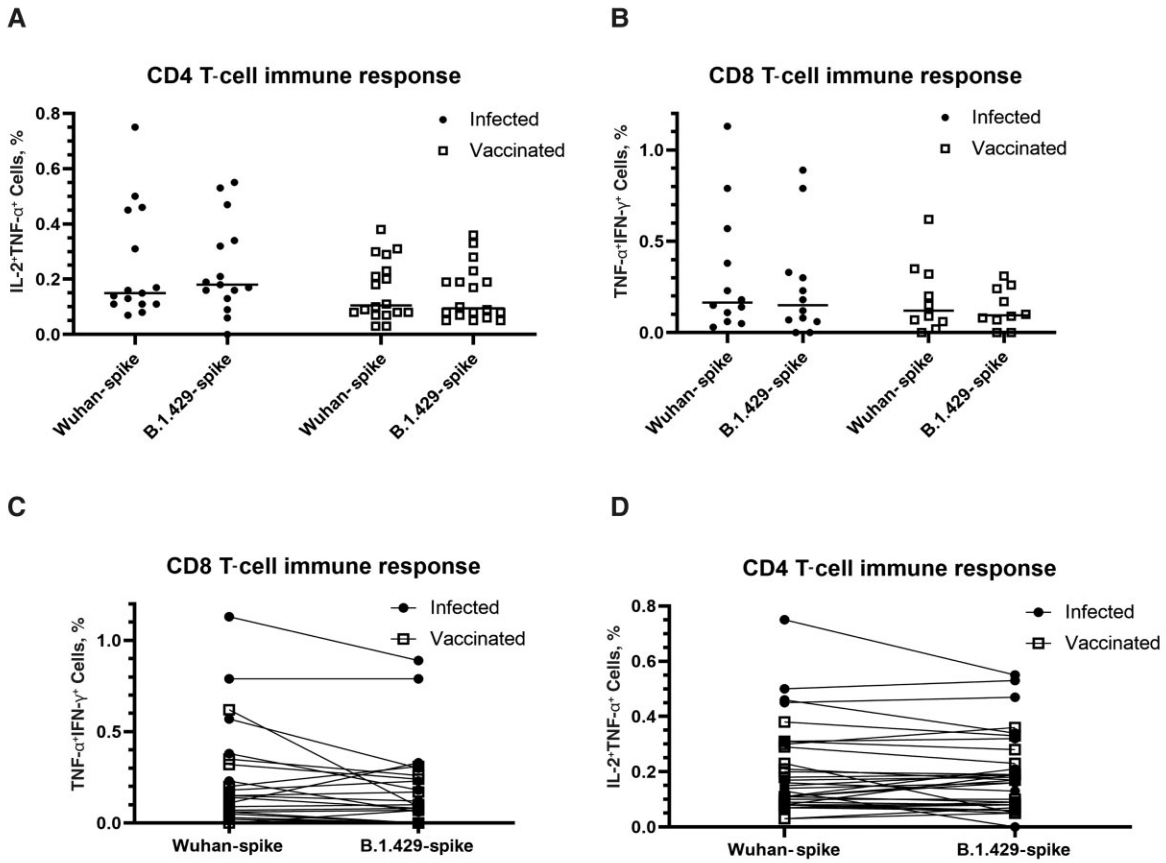

Figure 5. $CD4^+$ and $CD8^+$ T-cell responses to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) epsilon variant spike peptides, in infected and vaccinated individuals. T cells were stimulated by control (non-epsilon) or epsilon variant spike peptides. *A, B,* Activated $CD4^+$ (*A*) and $CD8^+$ T-cell (*B*) with dual cytokine expression in infected patients and vaccinated individuals. Each dot represents a single individual ($P > .05$). *C, D,* Paired data showing T-cell immune responses to the SARS-CoV-2 non-epsilon (control) and epsilon variant spike peptides for each individual ($P > .05$). Abbreviations: IFN, interferon; TNF, tumor necrosis factor.

vaccinated individuals elicited a comparable immune response against the epsilon and the non-epsilon variant spike proteins. Three infected patients and 8 vaccinated individuals had no detectable spike protein–specific $CD8^+$ T-cell immunity (data not shown). However, the other 12 infected patients and 10 vaccinated individuals had similar responses to epsilon peptides and non-epsilon spike protein (Figure 5*B*). T-cell reactivity was similar for both epsilon and non-epsilon spike peptides in all but 3 individuals. One individual lost $CD4^+$ reactivity and 2 lost $CD8^+$ reactivity against the epsilon spike protein (Figure 5*C* and 5*D*). Taken together, this indicates that the T-cell immune responses to the non-epsilon spike peptides generated after infection or vaccination with the BNT162b2 vaccine provide similar immune responses to the epsilon spike peptides.

DISCUSSION

Collectively, the current study demonstrates that live SARS-CoV-2 epsilon viral isolates mildly reduce the potency of antibody neutralizations. NAbs targeting the receptor-binding domain, seen in the epsilon variant, show a diminished effect, which is consistent with other reports of neutralizing antibodies [12] in unvaccinated individuals. Unlike previous reports of this variant [8], the response in vaccinated patients is slightly protective compared with that unvaccinated patients, suggesting an intact humoral response. This contradiction could stem the fact that previous studies using pseudoviral particles, while our current study used live viral exposure. In our study with live virus, we show that the viral kinetics, including plaque formation and results of proliferation assays, decrease with increased length of exposure to epsilon variant infection.

Although the L452R spike protein mutation increases transmission and infectivity [8, 12], these data provide promising information that T cells can co-opt their response over time when exposed to a more infectious variant, suggesting that there may be a lower risk of reinfection among patients previously infected by this COVID-19 variant. Certainly, viral replication and NAb differences seen in the exposure to live epsilon virus versus

pseudotype particles emphasize the need to consider the complete host response to any given SARS-CoV-2 variant in order to ascertain host resilience to mutations in a variant of concern or emerging variant. The tendency of SARS-CoV-2 to become more infectious but less virulent with the epsilon variant is supported mechanistically by (1) the down-regulation of viral processing pathways seen in our multiomic analyses and (2) the lack of associations with increased hospitalizations. In parallel, the cell's ability to adapt to this variant is seen in the decrease in cell proliferation over time and the alteration of metabolic mRNA processing at both RNA and protein levels.

The first mechanism of defense against any SARS-CoV-2 variant is the ability of T cells to respond efficiently, which is relatively unaltered in the epsilon variant. Consistent with this, data presented here suggest that patients infected with SARS-CoV-2 and those vaccinated with the BNT162b2 vaccine have comparable $CD4^+/CD8^+$ T-cell immune responses to the epsilon variant. This is not surprising, given data showing that 20%–50% of normal uninfected or vaccinated individuals have detectable T-cell responses to SARS-CoV-2 peptides, including spike protein [17–19]. These are engendered from previous exposures to seasonal coronaviruses with cross-reactive epitopes. Although the possibility is uncertain, these may provide an inside track for rapid deployment of immunity, which could result in a mild or asymptomatic clinical course.

The data presented by Peng et al [20] support the concept that there is a diverse and redundant immune response to multiple SARS-CoV-2 peptides, including Spike after infection. One of the critical observations from this study was the diversity of T-cell responses and their likely duration beyond antibody. Here we observe similar cross-reactivity with the epsilon variant. This suggests that T-cell diversity generated after SARS-CoV-2 infection or o vaccination will likely be characterized by diversity, cross-reactivity, and durability [20]. In support of these observations, Tarke et al [21] showed that all mutations in SARS-CoV-2 to date do not evade T-cell detection and also do not disrupt T-cell immunity; they suggested that the continued evolution of variants of concern highlights the critical importance of monitoring T-cell reactivity to ensure the integrity and persistence of immunity as the SARS-CoV-2 virus continues to mutate.

Despite an intact T-cell response to the epsilon variant, we observed an increase in mortality rate. While our data show that the T-cell response may be adapted to still be robust against the epsilon variant, if this primary immune response fails, our transcriptomic data from COVID-19 nasopharyngeal swab samples suggest that the innate immune system of patients with epsilon variants is significantly altered and perhaps not acting optimally in response to this variant. We also cannot rule out the possibility that such patients, once hospitalized, may be more prone to complications due to existing comorbid conditions (eg, diabetes mellitus) [22] and unrelated to their T-cell response. Finally, limitations to this analysis include the fact that all non-epsilon variants were compared with all epsilon variants; hence, other trends among variants of concern while they were emerging in this population were not captured.

Finally, our work indicates that, even with independent evolution, the mutations in the spike protein may converge and give rise to viral variants that display increased infectivity but also increased protective host-response. This holistic approach to evaluating emerging variants of concern and emerging variants could be an important concept in directing future vaccine development.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. J. T. P., S. J. C., E. V., J. E. V. E., and M. A. M. conceived of the project and experimental design. J. S. M. processed clinical samples. D. C., W. Z., A. B., S. S. C., B. D. D., A. S., S. K., and B. S. executed the experiments and generated the data. J. T. P., W. Z., A. B., R. Z., and F. D. conceived and executed all data analyses. S. S. C., the CORALE Study Group, A. B., and E. V. accessed and curated all clinical samples. W. Z., E. M., S. H., C. E., and E. V. accessed and analyzed all clinical data. All authors contributed to the writing of the manuscript.

Data availability. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE [23] partner repository, with the data set identifier PXD027995. Data can be accessed at ProteomeXchange [24].

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