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***TIGIT* Monoallelic Nonsense Variant in Patient with Severe COVID-19 Infection, Thailand**

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A heterozygous nonsense variant in the *TIGIT* gene was identified in a patient in Thailand who had severe COVID-19, resulting in lower *TIGIT* expression in T cells. The patient's T cells produced higher levels of cytokines upon stimulation. This mutation causes less-controlled immune responses, which might contribute to COVID-19 severity.

To investigate SARS-CoV-2 genomic variants, we recruited 46 COVID-19 patients from King Chulalongkorn Memorial Hospital in Bangkok, Thailand, in January 2020. Recruited patients were 16–79 years of age and had moderate to severe COVID-19 symptoms according to World Health Organization interim guidelines (<https://apps.who.int/iris/bitstream/handle/10665/331446/WHO-2019-nCoV-clinical-2020.4-eng.pdf>). We performed whole-exome sequencing on peripheral blood samples as described

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(1). The institutional review board of the Faculty of Medicine, Chulalongkorn University, Bangkok, approved this study (COA no. 738/2020).

We filtered variants by using the following criteria. Variants had to pass the quality standards, have read depth >10, and be from the coding regions or canonical splice sites of 1,810 immune-related genes, including immune checkpoint genes (2). Variants also had to have <1% allele frequency in the Genome Aggregation Database (gnomAD, <https://gnomad.broadinstitute.org>), Exome Variant Server (University of Washington, <https://evs.gs.washington.edu/EVS>), 1000 Genomes Project Consortium (<https://www.genome.gov>), dbSNPs (<https://www.ncbi.nlm.nih.gov/projects/SNP>), and Thai Reference Exome (T-Rex) database (3). We called candidate variants novel pathogenic variants when they were not previously identified in patients in the literature.

In our patient cohort, exome sequencing identified no variants in type I interferon genes, which previously have been commonly observed in patients with severe COVID-19 (4). Of note, we identified a heterozygous nonsense variant (rs1386709957) in the T-cell immunoglobulin and ITIM domain (*TIGIT*) gene in 1 patient (Appendix Figure 1, <https://wwwnc.cdc.gov/EID/article/29/11/22-0914-App1.pdf>). We did not identify this nonsense variant among 3,742 persons in the T-Rex database but did observe it in 1 of 31,390 alleles in the gnomAD database, in an allele from a female patient from East Asia. This variant truncates the 245-amino acid residue proteins at residue 56 and is classified as a pathogenic variant American College of Medical Genetics guidelines (<https://www.acmg.net>).

We investigated *TIGIT* gene expression in T cells of the patient from our study (Co45), a 43-year-old man, and compared it with 2 other sex- and age-matched patients who had severe COVID-19 (Co6 and Co84) (Appendix). We collected peripheral blood mononuclear cells (PBMCs) from each of the patients 1 month after they recovered. We used RNA extracted from PBMCs for real-time reverse transcription PCR and found patient Co45 had the lowest *TIGIT* mRNA level (Figure, panel A). Because *TIGIT* is mainly expressed in T cells, we used flow cytometry to measure the mean fluorescence intensity of *TIGIT* expressed in the cytoplasmic domain (CD) T cells. Patient Co45 had lower *TIGIT* gene expression in all CD3+, CD4+, and CD8+ T cells than the other 2 patients, most remarkably in the CD8+ T cells (Figure, panels B–D). The percentages of CD3+, CD4+, and CD8+ T cells in patient Co45 were comparable those in the other 2 patients (Appendix Fig-

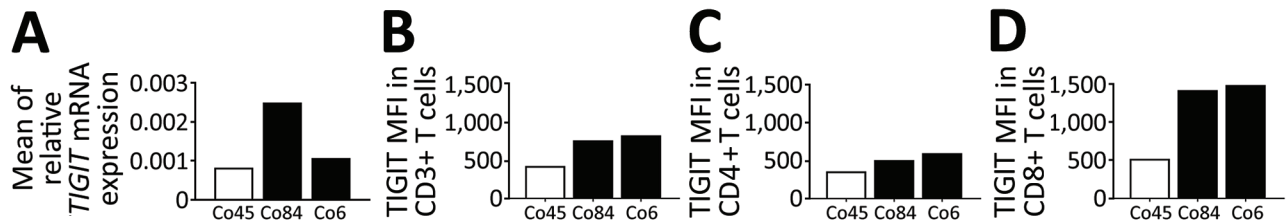


Figure. Results of rRT-PCR assay and flow cytometry of *TIGIT* nonsense variant in a patient with severe COVID-19 infection, Thailand. Co45 is the patient with *TIGIT* nonsense variant; Co84 and Co6 are age- and sex-matched patients who also had severe COVID-19 infection. A) Mean relative mRNA levels of *TIGIT* expression from rRT-PCR assay. B) *TIGIT* expression MFI on CD3+ T cells. C) *TIGIT* expression MFI on CD4+ T cells. D) *TIGIT* expression MFI on CD8+ T cells. CD, cytoplasmic domain; MFI, mean fluorescence intensity; rRT-PCR, real-time reverse transcription PCR; *TIGIT*, T cell immunoglobulin and ITIM domain gene.

ure 2, panel A), demonstrating that the truncated *TIGIT* variant reduced *TIGIT* expression in CD3+, CD4+, and CD8+ T cells.

TIGIT is known to exert immune suppressive functions, such as inhibiting T cell activation, proliferation, and functions that inhibit inflammation and anti-tumor responses. Thus, we investigated the effect of this monoallelic *TIGIT* variant on T cell functions by examining activation markers and cytokine-secreting T cells after stimulation with anti-CD3/CD28 coupled beads for 24 hours. We then assessed activation by using flow cytometry. We found no differences in frequencies of CD69-expressing CD3+, CD4+, and CD8+ T cells among the 3 patients (Appendix Figure 2, panel B); however, patient Co45 had higher interferon gamma (IFN γ), tumor necrosis factor alpha (TNF- α), and interleukin (IL) 2-producing CD3+, CD4+, and CD8+ T cells than the other 2 patients (Appendix Figure 3).

We believe this patient's heterozygous nonsense *TIGIT* variant contributed to the increased inflammatory cytokine functions we observed. His serum cytokine levels at acute illness onset did not differ from the other 2 COVID-19 patients (Appendix Figure 4), but some of his cytokine levels, including IL-10, IL-12p70, IL-4, and IL-7, remained high 1 month after recovery. Upregulation of co-inhibitory receptors, including cytotoxic T-lymphocyte-associated protein 4, programmed cell death protein 1, lymphocyte-activation gene 3, and T-cell immunoglobulin mucin-3, including *TIGIT*, has been reported in COVID-19 patients in other studies (5). These co-inhibitory receptors upregulated after T-cell activation to regulate immune responses and limit immunopathology (6,7). *TIGIT* can modulate expression of proinflammatory cytokines in acute lymphocytic choriomeningitis virus infection, in which the *TIGIT* blockage increased TNF- α expression by CD8+ T cells (8). *TIGIT*-deficient mice displayed increased IFN γ and IL-17+CD4+ T-cell frequencies (9). Simi-

larly, *TIGIT* knockdown can increase IFN γ expression in human T cells (10). We hypothesize that the nonsense *TIGIT* variant led to low *TIGIT* expression and hyperactive T responses in patient Co45 and might have contributed to his severe inflammation and symptoms. Unfortunately, the patient refused follow-up, so we could not perform further investigations to confirm our hypothesis.

In conclusion, we identified a patient with severe COVID-19 and a *TIGIT* monoallelic nonsense variant. He had lower *TIGIT* expression in CD3+, CD4+, and CD8+ T cells and produced higher cytokine expression, including IFN γ , TNF- α , and IL-2 upon stimulation. Our findings suggest *TIGIT* could be involved in COVID-19 severity.

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SARS-CoV-2 Omicron BA.1 Challenge after Ancestral or Delta Infection in Mice

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We assessed cross-reactivity to BA.1, BA.2, and BA.5 of neutralizing antibodies elicited by ancestral, Delta, and Omicron BA.1 SARS-CoV-2 infection in mice. Primary infection elicited homologous antibodies with poor cross-reactivity to Omicron strains. This pattern remained after BA.1 challenge, although ancestral- and Delta-infected mice were protected from BA.1 infection.

The SARS-CoV-2 Omicron variant (B.1.1.529, BA.1 sublineage) emerged nearly 2 years after the ancestral strain was identified (1). The Omicron BA.1 variant contains ≈50 mutations in the spike protein (2), resulting in substantial antigenic change. The strain was more infectious than prior variants of concern (VOCs) and escaped immunity, causing infections in persons who were previously vaccinated with ancestral strain-based vaccines (3) or infected with the ancestral virus or Delta (B.1.617.2) VOC. Since January 2022, additional Omicron sublineages (BA.2 to BA.5) have been detected worldwide. BA.4/BA.5 have identical spike proteins, most similar to BA.2, with additional spike mutations (4).

We sought to mimic the human scenario and selected a mouse model from available animal models (5) to assess the cross-reactivity of neutralizing antibody elicited by ancestral, Delta, and BA.1 viruses and to assess the effect of primary homologous and heterologous infection on secondary infection with the Omicron BA.1 strain. We also compared antibody cross-reactivity to BA.2 and BA.5 in serum samples from mice infected with ancestral, Delta, and BA.1 strains.

We first compared the associated illness, mortality rates, and kinetics of replication of 10^4 50% tissue culture infectious dose ($TCID_{50}$) of SARS-CoV-2/Australia/Vic/01/20 (ancestral strain-like),