ORIGINAL ARTICLE

CD4$^+$ T-Cell Dysfunction in Severe COVID-19 Disease Is Tumor Necrosis Factor-α/Tumor Necrosis Factor Receptor 1–Dependent

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Abstract

Rationale: Lymphopenia is common in severe coronavirus disease (COVID-19), yet the immune mechanisms are poorly understood. As inflammatory cytokines are increased in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, we hypothesized a role in contributing to reduced T-cell numbers.

Objectives: We sought to characterize the functional SARS-CoV-2 T-cell responses in patients with severe versus recovered, mild COVID-19 to determine whether differences were detectable.

Methods: Using flow cytometry and single-cell RNA sequence analyses, we assessed SARS-CoV-2-specific responses in our cohort.

Measurements and Main Results: In 148 patients with severe COVID-19, we found lymphopenia was associated with worse survival. CD4$^+$ lymphopenia predominated, with lower CD4$^+$/CD8$^+$ ratios in severe COVID-19 compared with patients with mild disease ($P < 0.0001$). In severe disease, immunodominant CD4$^+$ T-cell responses to Spike-1 (S1) produced increased *in vitro* TNF-α (tumor necrosis factor-α) but demonstrated impaired S1-specific proliferation and increased susceptibility to activation-induced cell death after antigen exposure. CD4$^+$TNF-α$^+$ T-cell responses inversely correlated with absolute CD4$^+$ counts from patients with severe COVID-19 ($n = 76$; $R = -0.797$; $P < 0.0001$). *In vitro* TNF-α blockade, including infliximab or anti-TNF receptor 1 antibodies, strikingly rescued S1-specific CD4$^+$ T-cell proliferation and abrogated S1-specific activation-induced cell death in peripheral blood mononuclear cells from patients with severe COVID-19 ($P < 0.001$). Single-cell RNA sequencing demonstrated marked downregulation of type-1 cytokines and NFκB signaling in S1-stimulated CD4$^+$ cells with infliximab treatment. We also evaluated BAL and lung explant CD4$^+$ T cells recovered from patients with severe COVID-19 and observed that lung T cells produced higher TNF-α compared with peripheral blood mononuclear cells.

Conclusions: Together, our findings show CD4$^+$ dysfunction in severe COVID-19 is TNF-α/TNF receptor 1–dependent through immune mechanisms that may contribute to lymphopenia. TNF-α blockade may be beneficial in severe COVID-19.

Keywords: COVID-19; SARS-CoV-2 infection; CD4$^+$ T cells; lymphopenia; TNF-α

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At a Glance Commentary

Scientific Knowledge on the Subject: Lymphopenia is common in severe coronavirus disease (COVID-19), and here we show it is associated with worse survival and is $CD4^+$ T-cell predominant. We further demonstrate that Spike-1 (S1) induces high concentrations of *in vitro* TNF-α (tumor necrosis factor-α) in $CD4^+$ T cells from patients with severe COVID-19 and further show impaired T-cell proliferation and increased susceptibility to activation-induced cell death. S1-specific $CD4^+TNF\text{-}\alpha^+$ T-cell responses inversely correlated with absolute $CD4^+$ counts from patients with severe COVID-19. *In vitro* TNF-α blockade, including infliximab or anti–TNF receptor 1 antibodies, strikingly rescued S1-specific $CD4^+$ T-cell proliferation and blocked S1-specific to activation-induced cell death in peripheral blood mononuclear cells from patients with severe COVID-19. Single-cell RNAseq demonstrated downregulation of type-1 cytokines and NFκB signaling in S1-stimulated $CD4^+$ cells with infliximab treatment. We further show that lung $CD4^+$ T cells also produce high concentrations of TNF-α in patients with severe COVID-19.

What This Study Adds to the Field: Together, our *in vitro* studies provide plausible TNF–α/TNF receptor 1-dependent immune mechanisms that may contribute to $CD4^+$ T-cell lymphopenia in severe COVID-19 and provide evidence that therapies targeting TNF-α may be beneficial in severe COVID-19.

Severe viral pneumonia and respiratory disease due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection have been the major clinical manifestation associated with patient mortality during the coronavirus disease (COVID-19) pandemic (1–3). The progression of upper respiratory symptoms to severe viral pneumonia, and at times the adult respiratory distress syndrome later in the course of infection in a subset of patients, have led many investigators to hypothesize an important role for inflammatory mediators or "cytokine storm" in the course of disease, as multiple studies have shown increased systemic levels of cytokines during moderate and severe COVID-19 disease (4, 5). Treatment with the corticosteroids dexamethasone and hydrocortisone was found to reduce mortality in severe COVID-19 pneumonia and suggested that immune modulation could impact severe disease (6, 7). Several subsequent studies of IL-6 receptor antagonists in severe COVID pneumonia have found mixed results, with some showing benefit (8–10) while others did not find efficacy (11–13). The recent NIH treatment guidelines recommend the use of tocilizumab in severe COVID-19 with rapidly decompensating respiratory status in combination with corticosteroids https://www.covid19treatmentguidelines.nih.gov/statement-on-tocilizumab/. The role of immune modulators in severe COVID-19 disease remains incompletely defined, as ongoing studies have not yet been completed.

An early report correlated lymphopenia with poor outcomes in COVID-19 and disease severity (14). However, the mechanisms leading to lymphopenia in COVID-19 clinical syndrome remain poorly understood (15). Recent studies of the peripheral T cells during SARS-COV-2 infection have found an activated phenotype in $CD8^+$ and $CD4^+$ T cells in severe disease, including increased surface expression of $CD38^+$, $CD95^+$, $HLA\text{-}DR^+$, $Ki67^+$, and PD-1, compared with mild disease and noninfected normal controls (16, 17). One study found that more profound lymphopenia was associated with increased serum concentrations of the inflammatory cytokines IL-6, IL-10, and TNF-α (tumor necrosis factor-α) (18). Another study found that IL-6 concentrations negatively correlated with cytotoxic immune cells in severe COVID-19 disease (19). We hypothesized that factors such as T-cell activation and the inflammatory milieu together contributed to the development of COVID–19-associated lymphopenia during severe disease. We further reasoned that other T-cell responses to SARS-CoV-2 proteins would be detected in mild and severe disease, in addition to spike, as earlier studies have demonstrated a broad response to SARS-CoV-2 epitopes across $CD4^+$ and $CD8^+$ T cells (20, 21). We hypothesized that SARS–CoV–2-specific T-cell responses might play an important role in the development of T-cell lymphopenia, as in other viral infections such as HIV that result in activation-induced cell death (AICD) (22).

Here, we found that COVID–19-associated lymphopenia in severe disease is disproportionately a $CD4^+$ T-cell lymphopenia and is associated with increased mortality, with significantly reduced peripheral $CD4^+/CD8^+$ T-cell ratios in severe disease compared with recently recovered patients with mild COVID-19. We further show that the immunodominant response of $CD4^+$ T cells is S1-specific production of the pro-inflammatory type-1 cytokine, TNF-α, in severe COVID-19 disease compared with patients with control mild COVID-19 disease. We observed impaired $CD4^+$ T-cell proliferation and AICD via TNFRI (TNF receptor 1) signaling that could be rescued *in vitro* with various TNF-α blockade agents. Similarly, reduced $CD4^+$ numbers and S1-specific TNF-α predominant responses were detected at higher frequencies in resident lung T cells from patients with recent severe COVID-19 pneumonia. Together, our findings show that $CD4^+$ T-cell dysfunction in severe COVID-19 respiratory disease is TNF–α/TNFRI-dependent.

Some of the results of these studies have been previously reported in the form of a preprint (bioRxiv, [03 June 2021] https://doi.org/10.1101/2021.06.02.446831).

Methods

Study Participants

Patients from the University of Pittsburgh Medical Center hospitals admitted with COVID-19 (moderate/severe disease on the basis of World Health Organization clinical progression scale [23]; $n = 148$) or outpatients who recently recovered within one month from mild COVID-19 ($n = 24$) screened for convalescent plasma donation were identified and provided informed written consent for participation in an

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

Institutional Review Board-approved protocol, at the University of Pittsburgh (STUDY20030236:COVID-19 Registry and Sample Repository) (Figure E1A in the online supplement). The patients were recruited from March 2020 through November 2020. Blood for functional immune studies was obtained within 2 weeks of hospitalization for the moderate/severe patients and 4–8 weeks from acute infection in the mild disease group. Of the moderate/severe COVID-19 subjects ($n = 148$), $n = 76$ had peripheral blood samples available for major immune analyses (T-cell subset frequencies and S1-specific flow cytokine assay). Clinical data, including demographics, labs, comorbidities, and patient survival, for these subjects were abstracted from the electronic health record. We randomly selected $n = 24$ moderate/severe samples and compared them with $n = 24$ mild recovered subjects for advanced immune studies in our functional immune cohort (surface and intracellular cytokine staining, ELISA assay for TNFα, *in vitro* antigen-specific T-cell proliferation with antibody blocking, bead depletion of $CD8^+$ T cells, negative selection for $CD4^+$ T-cell enrichment in peripheral blood mononuclear cells [PBMCs], AICD–apoptosis studies, and single-cell RNA sequence analysis [RNAseq]) (*see* supplemental methods).

Statistical Analysis

GraphPad Prism 9.1.2 and SPSS version 26 (IBM) were used for analysis. Cohort baseline characteristics were assessed using the chi-squared test for categorical data and the Mann-Whitney *U* test for continuous variables. For the hospitalized moderate/severe COVID-19 cohort, a time-to-event analysis comparing 30-day survival stratified by absolute lymphocyte count above or below the median was completed using Kaplan-Meier methods with the log-rank test. Additionally, Cox proportional hazards modeling was used to control for potential confounders. Age, sex, history of cardiovascular disease, history of chronic lung disease, and history of diabetes were screened for inclusion in a multivariable Cox regression. Variables with a P value ≤ 0.2 in univariable analysis and the multivariable regression were included in the final model. For the functional immune cohort (total $n = 48$), comparisons between the subgroup of moderate/severe COVID-19 and the mild recovered COVID-19 group, the Mann-Whitney *U* test for two-group comparisons, and the Friedman test followed by a *post hoc* test between each condition corrected for multiple testing or one-way multiple comparisons test for comparisons of more than two groups was performed. Two-way ANOVA was used for comparisons of more than two groups with two or more time points. Correlations were determined using the Spearman *P* test and Spearman rank correlation test. Using Boolean gating analysis and the Kruskal-Wallis one-way ANOVA test, the cytokine coexpression was determined using *SPICE* software (version 6.1), downloaded from http://exon.niaid.nih.gov/spice. For single-cell RNAseq analyses, we used CLC Genomics Workbench version 20, Seurat v3 and *R* software (*see* supplemental methods). P values < 0.05 were considered statistically significant.

Results

Peripheral Lymphopenia Is Associated with Mortality, with a Predominant $CD4^+$ T-Cell Lymphopenia in Severe COVID-19 Disease

We hypothesized that peripheral lymphopenia was associated with poor outcomes in patients with severe COVID-19 disease. We evaluated a multihospital cohort ($n = 148$) within our medical system of severe patients hospitalized with documented SARS-CoV-2 infection, with demographic data shown in Table E1. All patient selection criteria and clinical status are summarized in a consort diagram (Figure E1A). We evaluated the first absolute lymphocyte count (ALC) on hospitalization and found the median to be 700/mm^3 (Figure 1A). We next assessed 30-day mortality by Kaplan-Meier and observed increased mortality in those below the median (Figure 1B) and a trend for increased mortality in patients with $CD4^+$ lymphopenia (Figure 1C). We performed flow cytometry using a gating strategy on isolated PBMCs ($n = 76$ total) and detailed immune experiments in a functional cohort analyzed ($n = 48$) for both groups (severe = 24/mild = 24 patients with COVID-19) (Figures E1A and E1B; Tables 1 and E1). Patients ($n = 148$) whose first ALC obtained on admission was below the median maintained significantly reduced survival at 30 days in a Cox proportional hazards model controlling for age and history of diabetes (hazard ratio,1.85; 95% confidence interval,1.01–3.38) (Table E2). We observed a significant diminution in $CD4^+$ T-cell frequencies and reduced $CD4^+/CD8^+$ T-cell ratios in patients with lymphopenia (Figures 1D and 1F). We observed significantly reduced absolute $CD4^+$ and $CD8^+$ counts (though less profound) (Figures 1G and 1H) on the basis of ALC (Figure E1C). Together, these data indicate that COVID–19-associated lymphopenia is predominantly $CD4^+$ T-cell lymphopenia. To further evaluate T-cell function and phenotype, we randomly selected a subcohort of 24 patients with severe COVID-19, of which $n = 15$ (62.5%) had clinically-defined lymphopenia (Figure E1D), and a control cohort ($n = 24$) of mild COVID disease (Table 1) and $n = 15$ healthy controls (Figures 1I and 1J), immediately after resolution of symptoms and undergoing evaluation for potential plasma donation. While blood counts were not available for patients and healthy donors with mild, recovered COVID, we observed significantly higher $CD4^+/CD8^+$ ratios than our severe COVID-19 subcohort (Figures 1I and 1J).

$CD4^+TNF\text{-}\alpha^+$ and $CD4^+$ $CD107a^+$ Spike–1-Specific Effector Responses Are Immunodominant and Increased in Patients with Severe COVID-19

We next assessed COVID-specific and superantigen-induced T-cell effector responses (*Staphylococcal enterotoxin B*) in our mixed COVID-19 cohort (mild and severe disease; total $N = 48$). Using pooled peptides to the major SARS-CoV-2 antigens, we assessed both antigenic and effector immunodominance (major T-cell response) in PBMCs after a 6-hour *in vitro* restimulation. S1 responses (TNF-α and IFN-γ) were found to be immunodominant in $CD4^+$ T cells compared with the other antigens Spike-2, VEMP (viral envelope membrane protein), and nucleocapsid (Figures 2A and 2C). The S1 response seems to separate into two distinct clusters discriminated by the COVID-19 disease severity. A similar immunodominance pattern was found for COVID-specific $CD8^+IFN\text{-}\gamma^+$ T-cell responses (Figure E2A). We further compared blood S1-specific effector responses between $CD4^+$ and $CD8^+$ T cells in our severe COVID-19 cohort and found that S1-specific TNF-α responses predominated in $CD4^+$ T cells, in contrast to S1-specific $IFN\text{-}\gamma^+$ responses in $CD8^+$ T cells, but with similar frequencies of cytotoxic $CD107a^+$ responses (Figure 2D).

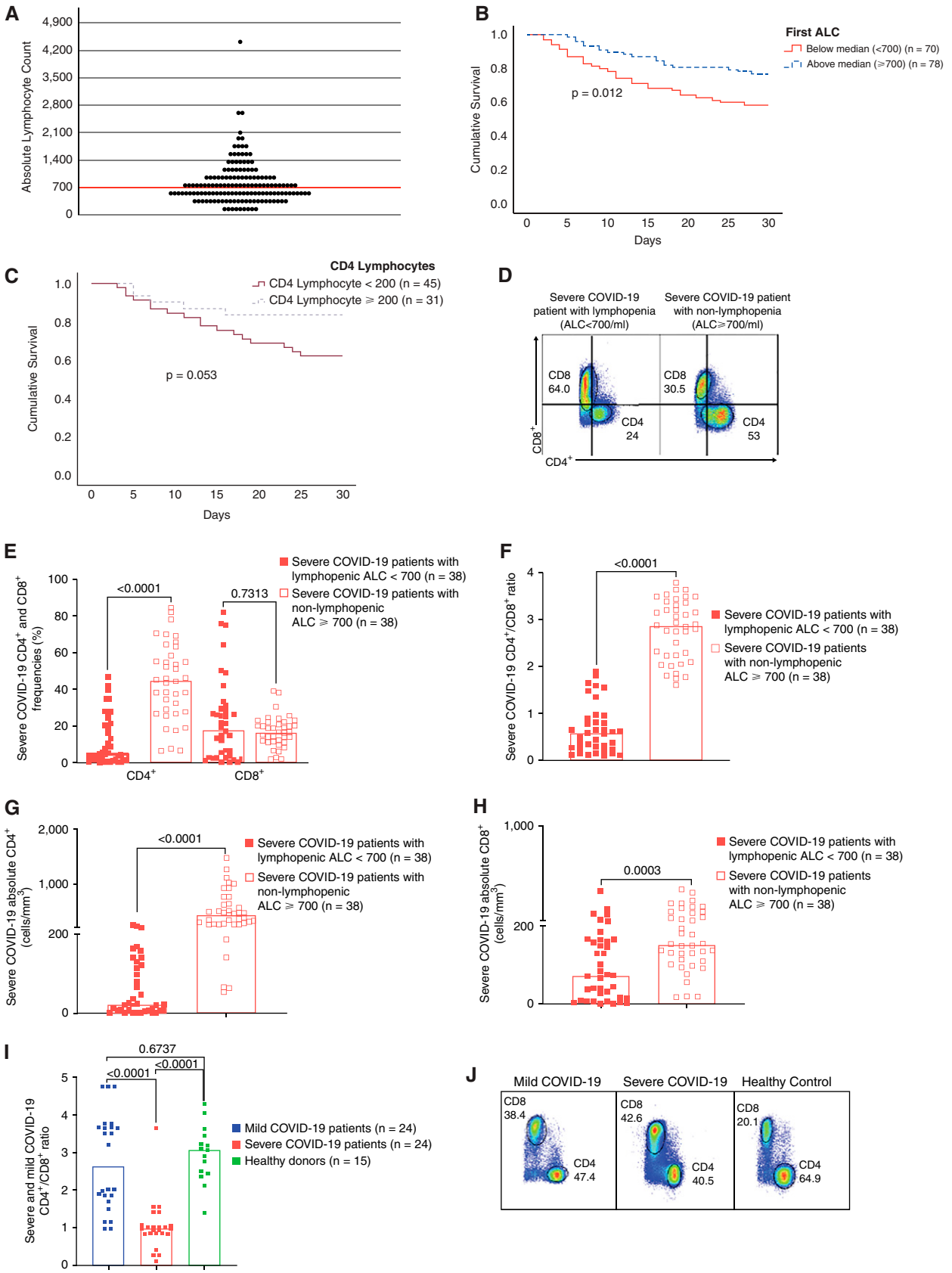

Figure 1. Peripheral lymphopenia is associated with mortality and a predominant $CD4^+$ T-cell lymphopenia in severe coronavirus disease (COVID-19). (*A*) The distribution of absolute lymphocyte counts (ALC) for $n = 148$ patients with severe COVID-19. Values represent the first ALC

Table 1. T-Cell Immunity Functional Cohort

	Moderate/Severe ($n = 24$)	Mild ($n = 24$)	*P* Value
Age, median (IQR)	69 (55–73)	45 (33–64.5)	<0.01
Race, n (%)	—	—	0.11
White	15 (62.5)	19 (79.2)	—
Black	5 (20.8)	2 (8.3)	—
Other	—	3 (12.5)	—
Unknown/no answer	4 (16.7)	—	—
Female, n (%)	11 (45.8)	10 (41.7)	0.77
Peak respiratory support, n (%)	—	—	—
Low-flow NC	1 (4.2)	—	—
High-flow NC	5 (20.8)	—	—
NIPPV	1 (4.2)	—	—
Mechanical ventilation	16 (66.7)	—	—
ECMO	1 (4.2)	—	—
Cardiovascular disease, n (%)	11 (45.8)	7 (29.2)	0.23
Respiratory disease, n (%)	2 (8.3)	5 (20.8)	0.42
Diabetes, n (%)	9 (37.5)	1 (4.2)	0.01

Definition of abbreviations: ECMO = extracorporeal membrane oxygenation; IQR = interquartile range; NC = nasal cannula; NIPPV = noninvasive partial pressure ventilation.
P values were calculated using the statistical analysis Fisher's exact test for categorical and Mann-Whitney *U* test for continuous and ordinal values.

The overall hierarchy of T-cell effector responses was found to be predominant type-1 immune responses, with little IL-17a or IL-13 COVID S1-specific responses detected.

We next determined whether there were differences adjusting for COVID-19 disease severity and found that S1-specific $CD4^+$ responses remained overall immunodominant and with higher frequencies in severe disease (Figure E2B), with $CD4^+TNF\text{-}\alpha^+$ and $CD4^+CD107a^+$ responses in particular increased among patients with severe COVID-19 compared with mild disease (Figure 2E). One exception was S1-specific $CD4^+IL\text{-}2^+$ frequencies which were significantly reduced among patients with severe compared with patients with mild disease. Notably, the hierarchy of $CD4^+TNF\text{-}\alpha^+ > IFN\text{-}\gamma^+$ responses in severe disease was observed with S1 and VEMP to a lesser extent, but not to other COVID antigens (Figure E2C). Conversely, S1-specific $CD8^+IFN\text{-}\gamma^+ > CD8^+TNF\text{-}\alpha^+$ cell responses were found to be significantly increased among those with severe versus mild disease ($P < 0.0001$), whereas $CD8^+CD107a^+$ responses did not differ ($P = 0.415$) (Figure 2F). Further, a comparison of S1-specific $CD4^+$ versus $CD8^+$ responses in severe disease shows higher $CD4^+TNF\text{-}\alpha^+$ frequencies, higher $CD8^+IFN\text{-}\gamma^+$ frequencies, and similar CD107a frequencies (Figure E2D). We next compared the multifunctionality of S1-specific $CD4^+$ versus $CD8^+$ effector T-cell responses. We found that $CD4^+$ T-cell multifunctional responses were significantly reduced in severe COVID disease compared with $CD8^+$ cells and with more $single^+CD4^+TNF\text{-}\alpha^+$ frequencies (Figure 2G). $CD4^+$ T cells demonstrated significantly reduced multifunctionality in patients with severe versus mild COVID-19, with reduced IL-2 and increased $CD4^+TNF\text{-}\alpha^+$ and $CD4^+CD107a^+$ frequencies (Figure 2H), in contrast to $CD8^+$ T-cell multifunctionality, which did not differ on the basis of COVID-19 disease severity (Figure E2E). We found that S1-specific $CD4^+TNF\text{-}\alpha^+$ response frequencies were inversely correlated with $CD4^+/CD8^+$ ratios in our functional COVID-19 cohort ($n = 48$) (Figure 2I).

Next, we measured S1-specific $CD4^+TNF\text{-}\alpha^+$ responses in our expanded number of patients with severe COVID ($n = 76$) and found this to be an inverse immune correlate of $CD4^+$ lymphopenia (Figure 2J) and $CD4^+/CD8^+$ ratios (Figure E2F). We measured plasma TNF-α (pg/ml) in our expanded number of patients with severe COVID ($n = 76$) and found this to be an inverse immune correlate with $CD4^+$ numbers (Figure E2G). In contrast, S1-specific $CD4^+IFN\text{-}\gamma^+$ or $CD4^+CD107a^+$ responses were not as strong an immune correlate with $CD4^+$ numbers (Figures E2H and E2I). Taken together, COVID-specific $CD4^+$ T cells are skewed in severe COVID-19 disease to produce high concentrations of TNF-α that inversely correlate with absolute $CD4^+$ counts.

Impaired S1-Specific $CD4^+$ T-Cell Proliferation in Severe COVID-19 Correlates with Lymphopenia, Is TNF–α/TNFRI-dependent, and Can Be Rescued *In Vitro* by Infliximab

Because we observed $CD4^+$ T-cell lymphopenia and reduced $CD4^+/CD8^+$ ratios among patients with severe COVID-19, we hypothesized that COVID-specific $CD4^+$ T-cell proliferative capacities contributed to low $CD4^+$ numbers. Therefore, we assessed S1-specific $CD4^+$ T-cell *in vitro* proliferation at 6 days after peptide restimulation and carboxyfluorescein succinimidyl ester (CFSE)-dilution and found significantly

Figure 1. (*Continued*). obtained upon admission and the median ALC (red line) was 700 cells/mm^3. (*B*) Kaplan-Meier 30-day survival curves for patients with severe COVID-19 with initial ALC values at or above the median (ALC ⩾ 700) ($n = 78$) versus those median (ALC <700) ($n = 70$), showing a significant difference in survival between groups (log-rank test, $P = 0.012$). (*C*) Kaplan-Meier 30-day survival curves on ($n = 76$) patients with severe COVID-19 with initial $CD4^+$ lymphocyte values ⩾200 ($n = 31$) versus those <200 ($n = 45$), showing a borderline difference in survival between groups (log-rank test, $P = 0.053$). (*D*) Representative flow cytometry plot of $CD4^+$ and $CD8^+$ T cells from patients with severe COVID-19 with lymphopenia ALC <700 (left panel) and nonlymphopenia ALC ⩾700 (right panel). (*E–H*) Cumulative data ($n = 76$) from patients with severe COVID-19 with lymphopenia (ALC <700) ($n = 38$) (solid red squares) compared with (*E*) nonlymphopenia (ALC ⩾ 700) ($n = 38$) (open red squares) showing frequencies of $CD4^+$ and $CD8^+$ T-cell subsets, (*F*) $CD4^+/CD8^+$ T-cell ratios, (*G*) the absolute $CD4^+$ T-cells number (cells/mm^3), (*H*) and $CD8^+$ T cell numbers (cells/mm^3). (*I*) Cumulative data showing $CD4^+/CD8^+$ T-cell ratio from the patient cohort used in functional studies ($n = 48$) with mild disease ($n = 24$) (blue squares) compared with severe disease ($n = 24$) (red squares) and with healthy donors ($n = 15$) (green squares). (*J*) Representative flow cytometry plots of $CD4^+$and $CD8^+$ T-cell frequencies from patients with mild (left panel) and severe COVID-19 (middle panel) and a healthy donor (right panel).

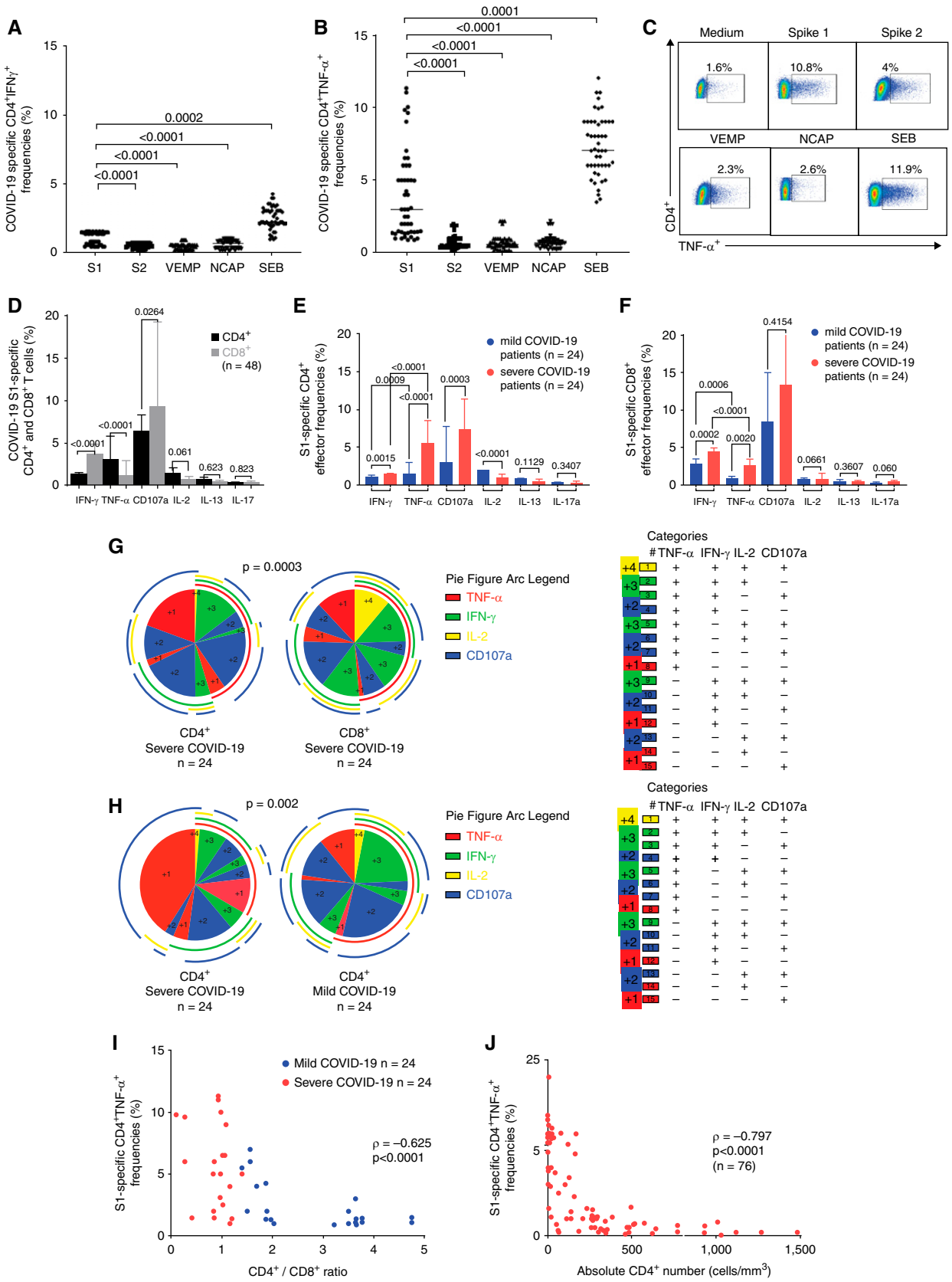

Figure 2. $CD4^+TNF\text{-}\alpha^+$ (tumor necrosis factor-α^+) and $CD4^+CD107a^+$ Spike-1 (S1)-specific effector responses are immunodominant and increased in patients with severe coronavirus disease (COVID-19). (*A* and *B*) Pooled data of patients with intracellular cytokine staining (ICS)

impaired proliferative responses in patients with severe COVID compared with mild disease (Figures 3A and 3B). While S1-specific $CD8^+$ T cell proliferative responses were also comparatively reduced in the severe disease cohort versus the mild disease cohort, impaired $CD4^+$ proliferation was more pronounced in severe disease, including in response to *S. enterotoxin B* (Figures E3A–E3D). Next, we evaluated whether differences in T-cell phenotype could account for impaired proliferation. We tested severe ($n = 24$) and mild ($n = 24$) COVID-19 patients and normal donors ($n = 15$) for cell surface CD45RA and CCR7 expression T cells and divided into central memory T cells ($CCR7^+$ $CD45RA^-$), effector memory T cells (CCR7− $CD45RA^-$), terminally differentiated effector T cells ($CCR7^-$ $CD45RA^+$), and naive ($CCR7^+$ $CD45RA^+$) cells (Figure E3E). We found that the $CD4^+$ percentages of memory, effector memory, terminally differentiated, and naive T cells were not significantly different between the groups.

As we detected reduced S1-specific IL-2 production from $CD4^+$ T cells in severe COVID-19, we determined whether exogenous IL-2 could rescue impaired proliferative responses. The addition of IL-2 to cultures significantly rescued S1-specific $CD4^+$ T-cell proliferation (Figures E3F and E3G) as well as enhanced S1-specific $CD8^+$ proliferative responses (Figure E3H). We next determined that $CD4^+$ proliferative responses correlated with $CD4^+/CD8^+$ ratios in the severe/mild COVID-19 functional cohort and within the severe disease cohort correlated with the ALC (Figures 3C and 3D). Taken together, our data support impaired $CD4^+$ T-cell proliferation as a mechanism for decreased $CD4^+$ counts in severe COVID-19 disease.

In Vitro TNFα/TNFRI Blockade Rescues Impaired S1-Specific $CD4^+$ T-Cell Proliferation

We next hypothesized that increased S1-specific TNF-α production from $CD4^+$ T cells negatively impacted proliferative capacities and may subsequently negatively impact $CD4^+$ T-cell numbers. To test this, we assessed the effect of TNF-α blockade on 12 patients with high TNFα responses and determined S1-specific $CD4^+$ proliferation at 6 days in PBMCs that were $CD8^+$ T–cell-depleted (96.2% $CD4^+$ purity) (Figures E3I and E3J) and found that TNF-α blockade resulted in significant restoration of $CD4^+$ proliferative responses (Figures 3E and 3F). We concomitantly measured TNF-α production from monocytes in the same cultures and found low TNF-α^+ frequencies after COVID-19 specific peptide restimulation (Figures E3K and E3L). Together, these data support $CD4^+$ T–cell-derived TNF-α as a significant source contributing to impaired S1-specific proliferation in severe COVID-19 disease.

Similarly, in the initial set of proliferation experiments in which $CD8^+$ T cells were not depleted, we observed enhancement of S1-specific $CD8^+$ proliferation in the presence of TNF-α blockade (Figure E3M). Next, we assessed TNFRI (CD120a) and TNFRII (CD120b) receptor surface expression in $CD4^+$ T cells across our cohort and found TNFRI surface expression was strikingly upregulated in $CD4^+$ T cells from patients with severe disease compared with mild disease controls and normal donors (Figures 3G and 3H). Targeted blockade of TNFRI resulted in the marked restoration of S1-specific $CD4^+$ proliferative responses, similar to TNF-α blockade, whereas TNFRII blockade had no significant effect (Figures 3I and 3J). Finally, we tested the impact of the anti-TNF–α therapeutic, infliximab, and found it capable of significantly rescuing S1-specific $CD4^+$ proliferation in a dose-dependent manner (Figures 3K and 3L); however, anti-PD1 blockade failed to rescue proliferation (Figure 3M). Moreover, infliximab *in vitro* treatment resulted in enhanced $CD4^+$IFN-γ, IL-2, and CD107a and reduced TNF-α responses at 6 days after secondary S1-peptide restimulation, thus reversing the hierarchical dominance of TNF-α in the type-1 response (Figures 3N and 3O). Together, our findings show TNF–α/TNFRI-dependent regulation of impaired $CD4^+$ proliferation and altered effector cytokines in severe COVID disease that can be reversed *in vitro* by infliximab.

S1 Induces Activation-induced Cell Death in $CD4^+$ T Cells from Patients with Severe Disease and Is TNF-α/TNFRI-Dependent

We further hypothesized that high COVID-specific TNF-α secretion from $CD4^+$ T cells might contribute to AICD and apoptosis, which may contribute to $CD4^+$ T-cell lymphopenia. To test this, we evaluated $CD4^+$ T cells for annexin V expression in short-term cultures (18h) after restimulation *in vitro* with S1 peptides in $CD8^+$-depleted cultures. We found that annexin V induction was significantly increased in patients with severe COVID compared with mild disease (Figures 4A and 4B). The addition of anti-TNF–α antibodies, including anti-TNFRI antibodies, significantly inhibited annexin V induction, as well as infliximab in a dose-dependent manner, but not anti-TNFRII antibodies (Figures 4C and 4D). We also found that anti-Fas or anti-TNF-related apoptosis-inducing ligand (anti-TRAIL) neutralizing antibodies inhibited annexin V induction in S1-activated $CD4^+$ T cells, but to a lesser extent than TNF-α blockade

Figure 2. (*Continued*). immunodominance ($n = 48$) used in our T-cell immunity functional cohort studies for COVID-19–specific S1, Spike 2 (S2), viral envelope small membrane protein (VEMP), nucleocapsid (NCAP), and staphylococcal enterotoxin B (SEB)-reactive of $CD4^+IFN\gamma^+$ (*A*) and $CD4^+TNF\alpha^+$ (*B*) effector responses. (*C*) Representative flow cytometry plots of ICS of severe patient $CD4^+TNF\alpha^+$ responses for medium, COVID-19–specific antigens S1, S2 (upper panels), VEMP, NCAP, and positive control–SEB (lower panels). (*D*) Cumulative data of ICS of S1-specific COVID-19 $CD4^+$ (black columns) and $CD8^+$ (gray columns) of T-cell effector responses (IFNγ, TNFα, CD107a, IL-2, IL-13, and IL-17) in ($n = 48$) patients used in functional studies. (*E* and *F*) Pooled data showing ICS of S1-specific COVID-19 $CD4^+$ (*E*) and $CD8^+$ (*F*) T-cell effector responses of IFNγ, TNFα, CD107a, IL-2, IL-13, and IL-17 in mild (blue columns) ($n = 24$) versus those with severe (red columns) ($n = 24$) COVID-19 disease. (*G* and *H*) Individual pie charts showing S1-specific multifunctional responses in severe COVID-19 of $CD4^+$ (left pie) and $CD8^+$ (right pie) ($n = 24$) (*G*) and $CD4^+$ in severe (left pie) ($n = 24$) versus $CD4^+$ in mild COVID-19 disease (right pie) ($n = 24$) (*H*). The T cell multifunctional responses were evaluated for four cytokines: TNFα (red arch), IFNγ (green arch), IL-2 (yellow arch), and CD107a (blue arch), and the combination of cytokine responses were: +4, yellow pie fraction; +3, green pie fraction; +2, blue pie fraction; and +1, red pie fraction. (*I*) Inverse correlation of S1-specific COVID-19 $CD4^+TNF\alpha^+$ response frequencies with $CD4^+/CD8^+$ ratio on patients with severe (red dots) and mild (blue dots) COVID-19 ($n = 48$). (*J*) The inverse correlation of S1-specific COVID-19 $CD4^+TNF\alpha^+$ response frequencies with absolute $CD4^+$ number (cells/mm^3) on severe COVID-19 ($n = 76$) (red dots).

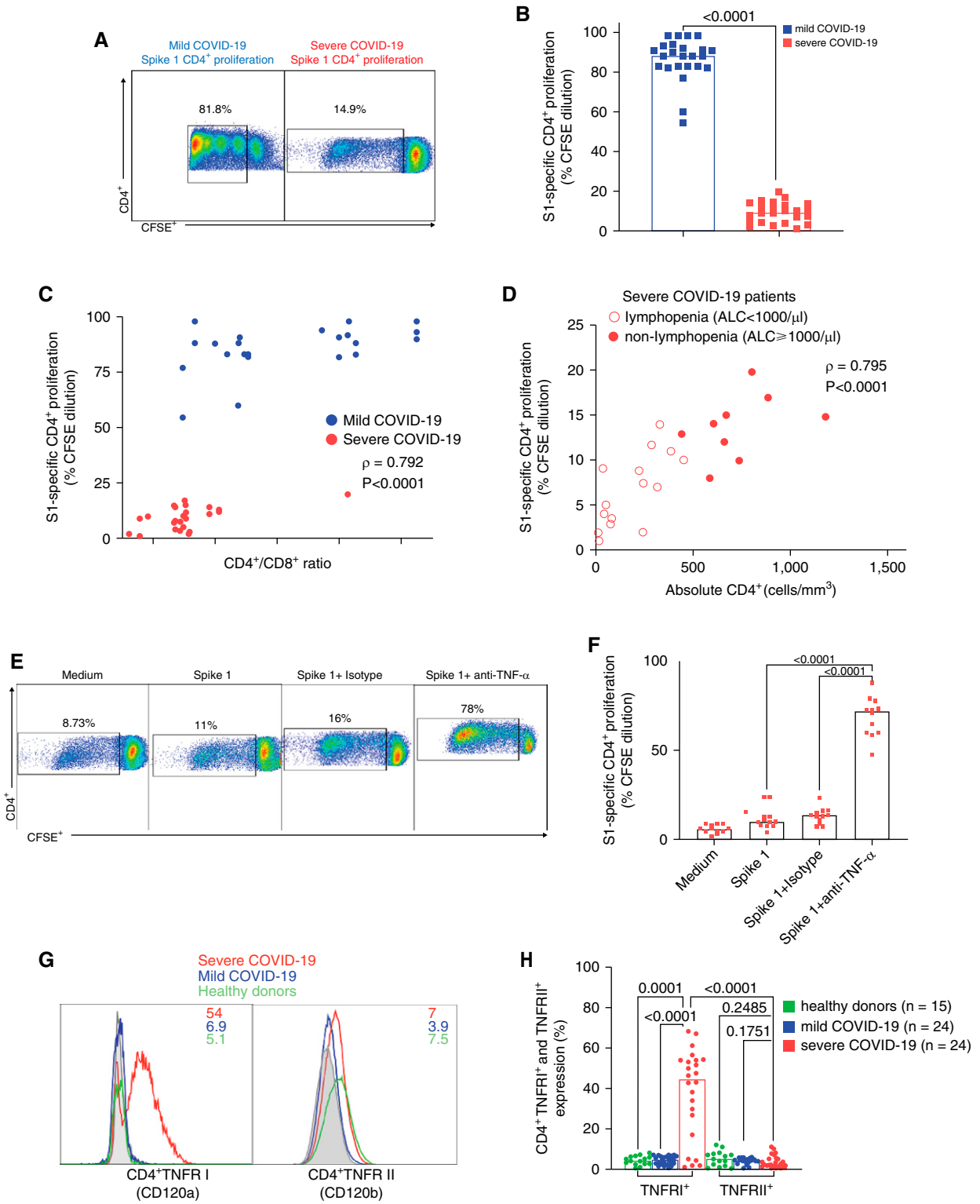

Figure 3. Impaired Spike-1 (S1)-specific CD4$^+$ T-cell proliferation in severe coronavirus disease (COVID-19) correlates with lymphopenia, is TNF-α (tumor necrosis factor-α)/TNFRI (TNF receptor 1)-dependent, and can be rescued *in vitro* by infliximab. (*A* and *B*) Representative flow cytometric plots (*A*) and cumulative data (*B*) showing Day 6 S1-specific CD4$^+$ T-cell proliferation by carboxyfluorescein succinimidyl ester (CFSE) dilution from patients with mild COVID-19 (left panel [*A*] and blue square [*B*]) versus severe (right panel [*A*] and red square [*B*]). (*C* and *D*) Direct correlation of S1-specific COVID-19 CD4$^+$ T-cell proliferation (Day 6) from mild (blue dots) and severe COVID-19 (red dots) ($n = 48$), response frequencies with CD4$^+$/CD8$^+$ ratio (*C*), and with absolute CD4$^+$ numbers in patients with severe COVID-19 with lymphopenia

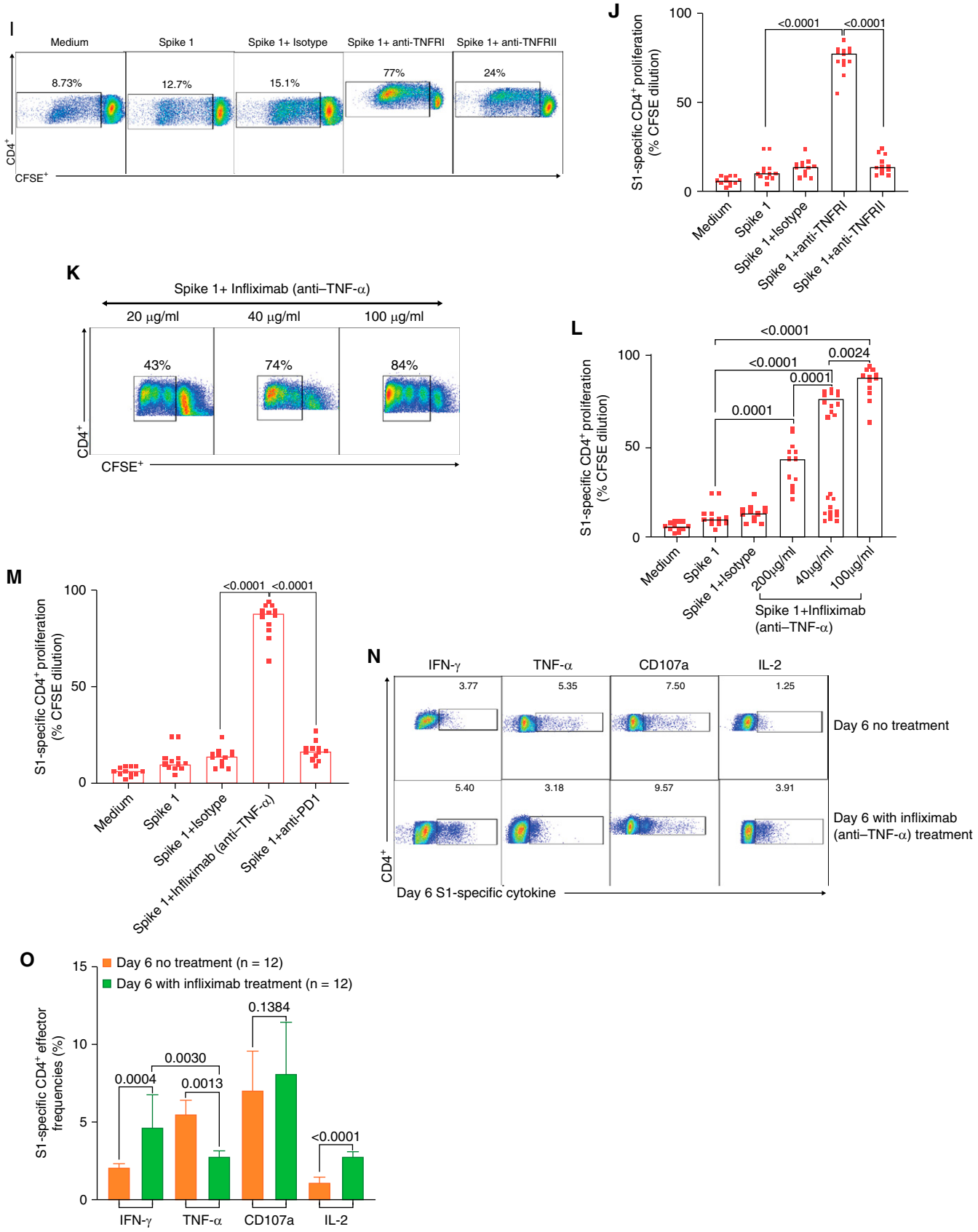

Figure 3. (*Continued*).

(Figures 4E–4H). Induction of AICD in cells from patients with severe disease occurred in the setting of other markers of activation, such as CD95, CD38, and to a lesser extent, PD-1, compared with mild disease or normal controls (Figure E5A). We observed that surface TNFRI expression correlated with CD38 expression (Figure E5B). Together, our data support S1-induced $CD4^+$ T-cell AICD in severe COVID disease is a predominantly TNF–α/TNFRI-dependent mechanism that may contribute to $CD4^+$ lymphopenia.

Single-Cell RNA Seq of S1-stimulated $CD4^+$ T Cells Reveals Striking TNF–α-Dependent Activation Blocked by Infliximab

We next examined the impact of TNF-α blockade on $CD4^+$ T-cell gene expression by performing single-cell RNAseq of peripheral $CD4^+$ T cells from two patients with severe COVID-19 (robust $CD4^+$ TNF-α production by flow cytometric studies) stimulated *in vitro* with S1 peptides in the presence or absence of infliximab. After negative selection-bead isolation of $CD4^+$ T cells, the single-cell RNAseq transcriptome identified seven distinct clusters (Figure E4A), largely differentiated by the presence or absence of anti-TNF–α blockade (Figures E4B and E4C). Those $CD4^+$ T cells exposed to S1 stimulation in the presence of infliximab demonstrated downregulation of genes related to pro-inflammatory cytokines (*IL2*, *IFNG*, *TNFa*), costimulation (*TNFSF14*[*LIGHT*] and *TNFSF*[*CD40LG*], NF-Kappaß signaling pathway[*NFKBID*], antigen binding and activation [*SLAMF1*], and apoptosis [*FASLG*, *MYC*, *BCL2A1*, *SELENOK*, *NR4A1*]) (Figures E4D and E4E). Gene set enrichment analysis of Hallmark gene sets identified upregulation of TNF signaling via NFKB, allograft rejection, and IL-2-STAT5 signaling pathways among the $CD4^+$ T cells stimulated with S1 alone (Figure E4F). Together, these single-cell RNAseq data indicate that TNF-α blockade using infliximab impacts transcripts in S1-stimulated $CD4^+$ T cells from two patients with severe COVID-19.

High TNF-α Production by Lung Resident Memory $CD4^+$ T Cells in Severe COVID-19 Disease

We next sought to determine whether $CD4^+$ T cells produced high TNF-α in the lung in severe COVID-19 disease. To do this, we evaluated explanted lung tissue from a 51-year-old male who underwent bilateral lung transplantation for end-stage fibrotic lung disease after severe COVID-19 infection. We assessed lung mononuclear cells from BAL fluid and lung parenchyma cells for $CD4^+$/$CD8^+$ ratios and S1-specific effector responses compared with PBMCs from this patient. Further, we assessed BAL versus PBMC S1-specific responses on five lung transplant recipients with documented SARS-CoV-2 infection, all of which had severe COVID-19 respiratory disease (Table E3). As seen in PBMCs from other severe COVID disease, the $CD4^+$/$CD8^+$ ratio was ⩽1 in the PBMC, BAL, and lung parenchyma (Figure 5A; Figure E5C). Increased $CD8^+$ T-cell numbers are consistent with a prior report on BAL cells from patients with severe COVID-19 (24). Compared with S1-specific responses from PBMC, $CD4^+$TNF-α > IFN-γ responses were significantly increased in the BAL and lung parenchyma (Figures 5B and 5C). Additionally, comparing $CD4^+$ versus $CD8^+$ cells, BAL $CD4^+$TNF-α^+ frequencies were higher, $CD8^+$IFN-γ^+ frequencies were higher, and $CD107a^+$ frequencies were similar (Figure E5D). Lung $CD4^+$ T cells demonstrated $CD69^+CD103^{+/-}CD45RA^-CCR7^-$ resident memory phenotype, with increased $CD38^{hi}PD1^{hi}Ki67^{hi}$ activation phenotype (Figures 5D and 5E). Lung $CD8^+$ T cells were evaluated and demonstrated $CD69^+CD103^{+/-}CD45RA^-CCR7^-$ resident memory phenotype (Figures E5E and E5F). Together, these data support reduced $CD4^+$ T cell numbers in the lung with severe COVID-19 and increased S1-specific TNF-α production from activated $CD4^+$ resident memory T cells.

Discussion

Herein, we show that severe COVID–19-associated lymphopenia is a predominant $CD4^+$ lymphopenia and is associated with an increased risk for mortality. Overall, severe COVID-19 disease was associated with a significant diminution in the peripheral $CD4^+$/$CD8^+$ ratios, whereas patients who recovered from mild COVID-19 demonstrated preservation of normal $CD4^+$/$CD8^+$ ratios. On the basis of these findings, we assessed the function of peripheral $CD4^+$ T cells from patients with severe COVID-19 versus patients with mild recovered COVID-19 to evaluate immune mechanisms driving $CD4^+$ lymphopenia. Indeed, we found a disproportionate increase in TNF-α production and cytotoxic function from $CD4^+$ T cells, but not $CD8^+$ T cells, in response to the immunodominant antigen, S1, evident in severe COVID-19 disease. We also found that S1-specific TNF–α-dependent production from $CD4^+$ T cells themselves and enhanced TNF-α responsiveness via TNFRI are key mechanisms leading to impaired $CD4^+$ proliferation and AICD and contribute to $CD4^+$ lymphopenia. Together, our findings point to a skewed pro-

Figure 3. (*Continued*). (open red dots) and nonlymphopenia (solid red dots) ($n = 24$) (*D*). (*E* and *F*) Representative flow cytometric plots (*E*) and cumulative data (*F*) showing S1-specific $CD4^+$ T-cell proliferation from $CD8^+$-depleted peripheral blood mononuclear cells (PBMC) in patients with severe disease ($n = 12$) (red squares) in the presence or absence of anti–TNF-α antibodies. (*G* and *H*) Representative histogram plots (*G*) and cumulative data (*H*) showing $CD4^+$ T-cell $TNFRI^+$ (CD120a) (left panel [*G*]) and $TNFRII^+$ (CD120b) (right panel [*G*]) surface expression in mild ($n = 24$) COVID-19 (blue lines [*G*] and blue dots [*H*]) overlayed with severe COVID-19 (red lines [*G*] and red dots [*H*]) ($n = 24$) and normal healthy donors (green lines [*G*] and green dots [*H*]) ($n = 15$) and the isotype control (full gray lines [*G*]), and the numbers represent the percentage of positive expression of TNFRI and TNFRII. (*I* and *J*) Representative flow cytometric plots (*I*) and cumulative data (*J*) of $CD4^+$ S1-specific proliferation in the presence or absence of anti-TNFRI or anti-TNFRII antibodies in $CD8^+$-depleted PBMC from patients with severe COVID-19 (red squares) ($n = 12$). (*K–M*) Representative flow cytometric plots (*K*) and cumulative data (*L*) showing $CD4^+$ S1-specific proliferation in the presence or absence of infliximab at various doses and the presence or absence of anti-PD1 antibodies (*M*) and isotype control (IgG1) (red squares). (*N* and *O*) Representative flow cytometric plots (*N*) and cumulative data (*O*) showing S1-specific frequencies of $CD4^+$ T-cell effector responses (IFNγ, TNFα, CD107a, and IL-2) after 6 h restimulation with S1 peptides and infliximab (lower panel [*N*], green columns [*O*]) or without infliximab treatment (upper panels [*N*], orange columns [*O*]) for patients with severe COVID-19 ($n = 12$).

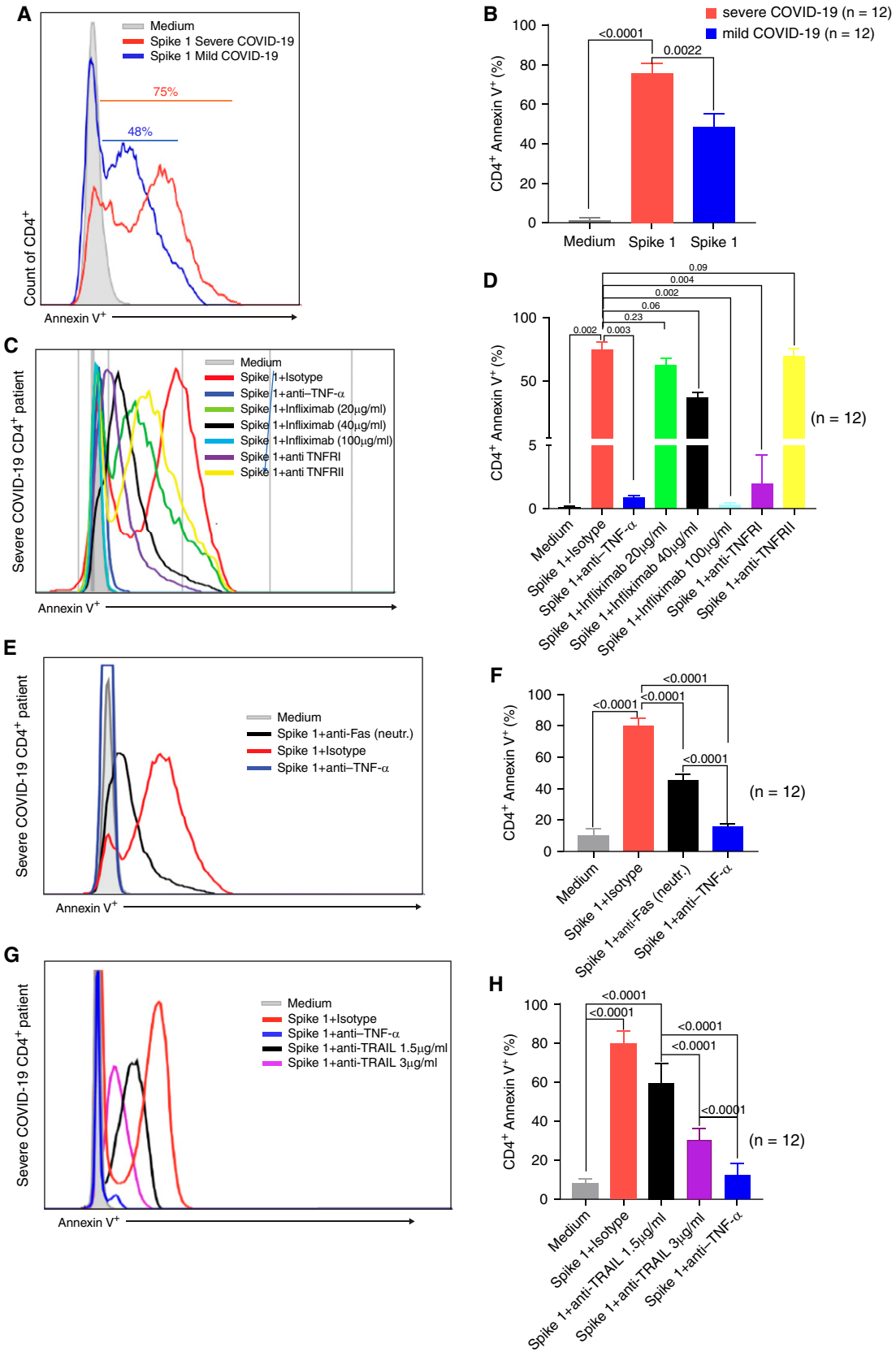

Figure 4. Spike-1 (S1) induces activation-induced cell death in $CD4^+$ T cells from patients with severe disease and is TNF-α (tumor necrosis factor-α)/TNFRI (TNF receptor 1)-dependent. (*A* and *B*) Representative histograms overlayed from patients with severe (red line) versus mild

inflammatory $CD4^+$ T-cell response among patients with severe COVID-19 that is central to $CD4^+$ T-cell dysfunction and that plausibly contributes to the immunopathogenesis of severe disease.

While our studies found that S1 is the immunodominant antigen for SARS–CoV–2-induced T-cell responses, we also detected significant effector T-cell responses to other viral antigens, namely Spike-2, nucleocapsid, and VEMP. However, $CD4^+$ T-cell responses to these antigens from patients with severe disease did not demonstrate a hierarchical dominance of TNF-α to these other SARS-CoV-2 antigens compared with S1, whereas CD107a responses were similarly elevated to all antigens with severe disease. Further, S1 was also immunodominant for $CD8^+$ T-cell responses; however, IFN-γ and CD107a responses were the highest frequencies in severe disease in contrast to TNF-α and CD107a having the highest frequencies in $CD4^+$ responses. Unexpectedly, S1-specific CD107a responses were similar between $CD4^+$ and $CD8^+$ responses in severe disease, whereas $CD4^+$TNF-α responses were comparatively higher in severe disease and IFN-γ responses were higher in $CD8^+$ T cells. Lastly, we show that $CD4^+TNF^+$ responses had the highest inverse correlation ($P = -0.797$) with absolute $CD4^+$ T-cell counts in severe disease. Together, these findings reveal that the immunodominant SARS-CoV-2 S1 protein elicits strong TNF-α and CD107a cytotoxic responses from $CD4^+$ T cells.

Earlier studies on HIV, measles, and dengue viral infections have demonstrated that viremia results in impaired $CD4^+$ T-cell proliferation (25–27). While we cannot exclude SARS-CoV-2 replication impacting the reduced $CD4^+$ proliferative responses observed in severe COVID-19 disease, we demonstrate that *in vitro* TNF-α blockade using infliximab, anti-TNFRI, or other TNF-α antibodies strikingly restored these responses. Moreover, depleting $CD8^+$ T cells and our finding of minimal spontaneous or S1-induced TNF-α from monocytes supports the concept that $CD4^+$TNF-α production plays a major role in driving $CD4^+$ dysfunction. Prior studies have shown mixed results on the impact of TNF-α on T-cell responses. TNF-α can act as a costimulatory molecule and enhance T-cell receptor-dependent activation of T cells leading to increased cytokine and proliferative responses via NFκB signaling (28–31). These costimulatory effects of TNF-α on T-cell responses are predominantly mediated through TNFRII (32, 33). However, other studies have demonstrated *in vivo* that chronic exposure to TNF-α results in prolonged survival and attenuation of disease in NZB1/W F1 mice prone to lupus nephritis and suppresses type I insulin-dependent diabetes mellitus in nonobese diabetic mice (34, 35). Indeed, Cope and colleagues demonstrated that chronic TNF-α exposure inhibited T-cell proliferation and cytokine production through TNFRI (p55-receptor) and that *in vitro* proliferative and type-1 cytokine responses could be rescued using anti-TNF–α therapy, consistent with our observations (36). Interestingly, several reports have shown increased IFNγ T-cell responses in patients on infliximab therapy, consistent with our findings on Day 6 *in vitro* (37–40). In contrast to our findings, other studies have found a T-cell inhibitory role of T-cell–receptor-dependent activation through TNFRII and chronic TNF-α exposure (41). Additionally, chronic TNF-α exposure-induced T-cell hyporesponsiveness was subsequently shown to lead to impaired NF-KB signaling (42). Thus, differential effects of TNF-α on T-cell responses and T–cell-mediated pathology have been observed depending on the model system and duration of cytokine exposure. Our findings suggest that severe COVID-19 infection with persistent S1-specific TNF-α production from $CD4^+$ T cells more closely models chronic TNF-α exposure, resulting in T-cell hyporesponsiveness and dysfunction.

In addition to anti-TNF–α blockade agents, we found that low-dose exogenous IL-2 also rescued S1-specific proliferative responses, and we observed that S1-specific $CD4^+$IL-2 frequencies significantly increased after 6 days in the presence of TNF–α-blockade. Together, these data point to the preservation of IL-2 responsiveness and support reduced $CD4^+$IL-2 frequencies in severe COVID-19 disease that could be enhanced *in vitro* with TNF-α blockade.

TNF-α can be a major regulatory pathway for apoptosis of various cells, as well as cell survival (28, 43). While the TNF-α/TNRI pathway has been demonstrated to be the predominant pathway for apoptosis in T cells, there is also evidence that the TNFRII plays a role (44). T-cell apoptosis via the TNFRI and/or TNFRII has been shown to be an important regulatory mechanism for the T-cell response in viral infections such as lymphocytic choriomeningitis virus and influenza, as well as autoreactive and aged T cells (45–48). The TNF-α/TNFR pathways play highly complex direct and indirect roles in $CD4^+$ and $CD8^+$ T-cell apoptosis during HIV infection that include both TNFRI and TNFRII, in addition to other mechanisms

Figure 4. (*Continued*). (blue line) coronavirus disease (COVID-19) and medium as control (gray full line) (*A*) and pooled data depicting the annexin V^+ staining in $CD4^+$ T cells in patients with severe ($n = 12$) (red column) versus mild ($n = 12$) (blue column) COVID-19 versus medium (gray column) (*B*). (*C* and *D*) Representative histograms (*C*) and pooled data (*D*) showing the $CD4^+$annexin V^+ in $CD8^+$-depleted peripheral blood mononuclear cells from patients with severe COVID-19 ($n = 12$). Cells were cultured in the presence or absence of anti–TNF-α neutralizing antibodies (dark blue histogram lines [*C*] and dark blue column [*D*]), infliximab at various doses (green, black, and light blue histogram lines [*C*] and columns [*D*]), anti-TNFRI antibodies (purple histogram lines [*C*] and purple column [*D*]) or anti-TNFRII (yellow histogram lines [*C*] and yellow column [*D*]) antibodies, compared with S1-specific isotype control (IgG1) (red histogram lines [*C*] and red column [*D*]) or medium alone (gray full histogram lines [*C*] and gray column [*D*]). (*E* and *F*) Representative histograms (*E*) and pooled data (*F*) showing the $CD4^+$annexin V^+ from patients with severe COVID-19 in the presence or absence of anti-Fas neutralizing antibodies (black histogram lines [*E*] and black column [*F*]), compared with anti–TNF-α neutralizing antibodies (dark blue histogram lines [*E*] and dark blue column [*F*]) versus S1-specific isotype control (IgG1) (red histogram lines [*E*] and red column [*F*]) and medium as control (gray full line [*E*] and gray column [*F*]). (*G* and *H*) Representative histograms (*G*) and pooled data (*H*) depicting the $CD4^+$annexin V^+ from patients with severe COVID-19 in the presence or absence of anti–TNF-related apoptosis-inducing ligand (TRAIL) neutralizing antibodies at various doses (black and purple histogram lines [*G*] and black and purple columns [*H*]), compared with anti–TNF-α neutralizing antibodies (dark blue histogram lines [*G*] and dark blue column [*H*]), versus S1-specific isotype control (IgG1) (red histogram lines [*G*] and red column [*H*]) and medium as control (gray full line [*G*] and gray column [*H*]).

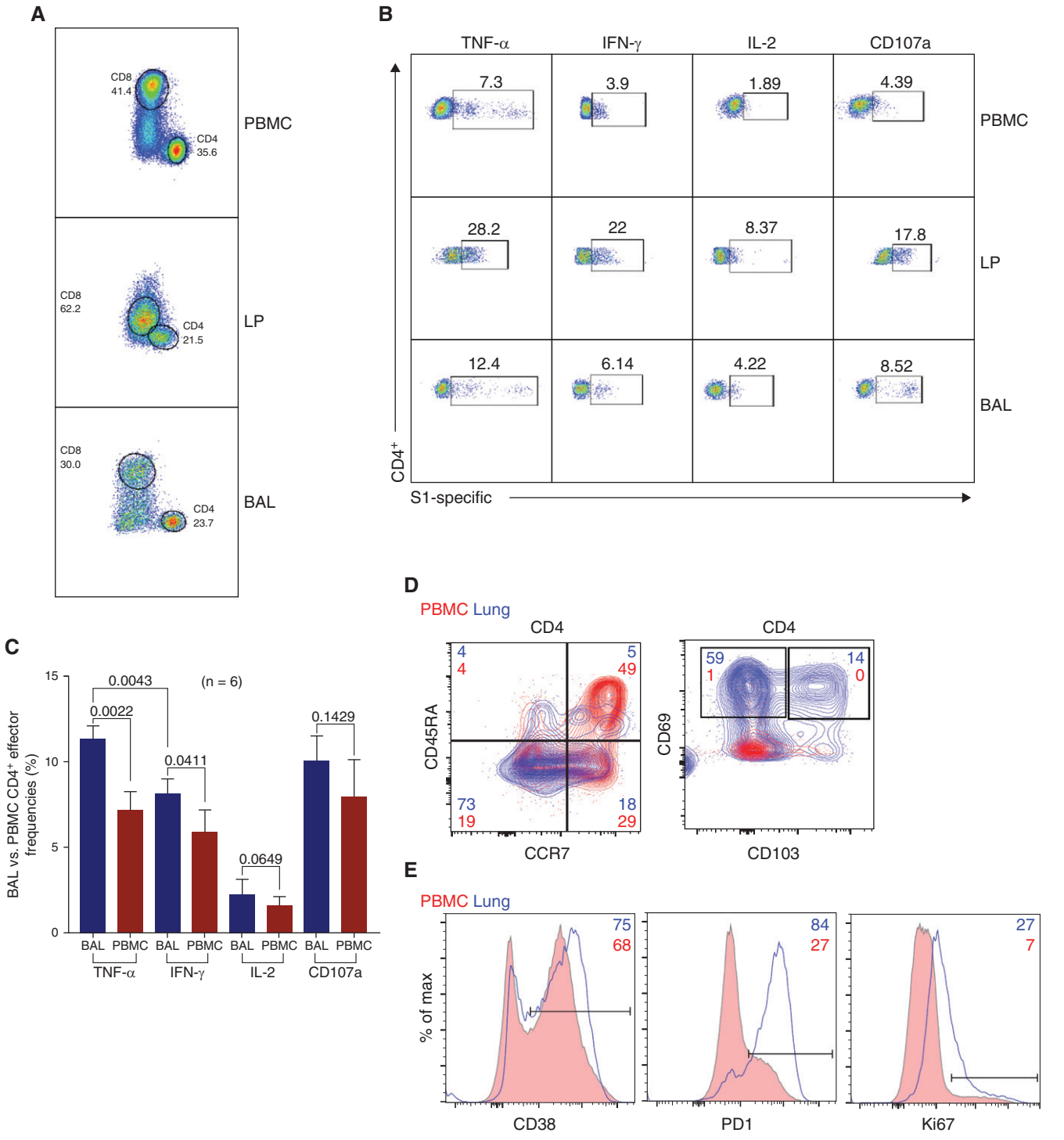

Figure 5. High TNF-α (tumor necrosis factor-α) production by lung resident memory $CD4^+$ T cells in patients with severe coronavirus disease (COVID-19). (*A*) Representative flow cytometry plots on explanted lung tissue in patients with severe COVID-19 of $CD4^+$ and $CD8^+$ T-cell frequencies in peripheral blood mononuclear cells (PBMC) (upper panel); lung parenchyma (LP) (middle panel), and BAL fluid–derived cells (lower panel). (*B*) Representative flow cytometry plots of the same patient with COVID-19 showing $CD4^+$ T-cell frequencies at 6 h after Spike-1 (S1)-specific peptides stimulation for TNF-α, IFN-γ, IL-2, and CD107a in PBMC (upper panels); LP (middle panels), and BAL fluid–derived cells (lower panels). (*C*) Pooled data showing intracellular staining of 6 h S1-specific $CD4^+$ T-cell effector responses: TNF-α, IFN-γ, IL-2, and CD107a in BAL fluid-derived cells from $n = 6$ as lung transplant recipients (dark blue columns) versus PBMC (dark red columns). (*D*) Representative flow cytometry plots of the COVID-19 lung explant showing $CD4^+$ T-cell surface expression of $CD45RA^+$, $CCR7^+$ (left panel), and $CD69^+CD103^+$ (right panel) on PBMC (red) overlayed with LP (blue) lung; values represent cell frequencies of T-cell subsets. (*E*) Flow cytometry histograms of the COVID-19 explant showing LP lung $CD4^+$ T cells $CD38^+$ (left panel), $PD1^+$ (middle panel), and $Ki67^+$ (right panel) overlayed with LP lung (blue lines) on PBMC (full pink lines).

(49, 50). A recent study showed that the combination of TNF-α and IFN-γ drives inflammatory cell death, PANoptosis, and induces the cytokine storm (51). Our studies show that TNF–α/TNFRI-dependent apoptosis of $CD4^+$ T cells via AICD is a major mechanism contributing to $CD4^+$ lymphopenia in severe SARS-CoV-2 infection. We also observed that TNFRI-mediated apoptosis in $CD4^+$ T cells in severe COVID-19 was associated with increased surface expression of other activation markers such as CD95(Fas) and CD38, and to a lesser extent PD-1, as previously reported in HIV infection (52, 53).

We also observed that blockade of the TRAIL or Fas/FasL pathway, the major apoptotic pathway in HIV infection, also rescued $CD4^+$ T cells from AICD, though to a lesser extent than TNF-α (22, 54). Our RNAseq studies also demonstrated that TNF–α-blockade significantly reduced the expression of type-1 cytokines, including TNF-α itself, NFκB-signaling, and FasL, consistent with the significant rescue of S1-restimulated $CD4^+$ T cells from AICD. Taken together, our findings support high concentrations of TNF–α/TNFRI-dependent apoptosis of $CD4^+$ T cells through AICD in severe COVID-19 disease that contributes to lymphopenia. We evaluated lung resident T cells from an explanted lung (severe COVID-19) undergoing lung transplantation and found a similar diminution in the $CD4^+$/$CD8^+$ ratio in lung parenchymal and BAL cells, similar to PBMC. We further assessed BAL samples from six lung transplant recipients (LTRs) with recent severe COVID infection and had similar findings of reduced $CD4^+$ frequencies in the lung, along with increased $CD4^+$ S1-specific TNF-α production, which was increased in both the lung parenchyma and BAL compartments compared with the PBMC. These findings are consistent with our previous findings of enhanced $CD4^+$ effector T-cell responses in the lung during acute and chronic cytomegalovirus infection but unusual in their TNF-α predominance (55). Together, our findings support the role of increased TNF-α production from both $CD4^+$ and $CD8^+$ T cells in contributing to lung inflammation in severe COVID-19 disease and reduced $CD4^+$ numbers in the lung (56).

Our studies found that the TNF-α blockade therapy, infliximab, a common therapy for rheumatoid arthritis and other autoimmune diseases, had a profound dose–response *in vitro* effect in the restoration of S1-specific $CD4^+$ proliferative responses and abrogation of S1-induced AICD (57). Indeed, recent studies have shown that treatment with the IL-6R antagonist tocilizumab resulted in increased circulating lymphocytes and cytotoxic NK cells (19, 58). Moreover, high IL-6 and TNF-α concentrations on hospital admission correlated with mortality in severe COVID-19 disease (59). Recently, two clinical studies observed improved COVID-19 outcomes with TNF-α inhibition (infliximab) in patients with severe COVID-19 (60, 61). A current NIH phase 3 trial is evaluating infliximab along with other immunomodulators (ACTIV-1 IM trial) for moderate to severe COVID-19 disease; ACTIV-1 IM is a master protocol designed to evaluate multiple investigational agents for the treatment of moderately or severely ill patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Together, our studies and other lines of evidence suggest a potential role for TNF-α blockade therapy in severe COVID-19 respiratory disease.

Conclusions

We show that the $CD4^+$ T-cell immunodominant response to S1 during severe COVID induces high TNF-α concentrations resulting in TNF-α/TNFRI–dependent mechanisms of impaired $CD4^+$ proliferation and susceptibility to AICD, where $CD4^+$-derived TNF-α may play an important role. We further found that high $CD4^+$TNF-α production was inversely correlated with reduced absolute $CD4^+$ numbers during severe COVID disease and show that lymphopenia is associated with increased mortality. Importantly, lung resident $CD4^+$ T cells produce elevated TNF-α along with reduced $CD4^+$/$CD8^+$ T-cell ratios in severe disease. Together, our studies demonstrate plausible TNF-α/TNFRI–dependent mechanisms that may lead to COVID–19-associated lymphopenia and provide a strong rationale for testing TNF-α blockade therapy in severe COVID-19 disease. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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