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Dynamic changes in the immunological characteristics of T lymphocytes in surviving patients with severe fever with thrombocytopenia syndrome (SFTS)

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ABSTRACT

Objective: Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease with high mortality. T cell deficiency has recently been described, but the changes in T cell functionality during acute SFTS virus (SFTSV) infection and the mechanisms leading to T lymphocyte death remain largely unknown. This study was conducted to evaluate T cell functionality and the expression of apoptotic/proliferation and activation/inhibition markers during acute SFTSV infection.

Methods: Twenty-eight surviving SFTS patients were sequentially sampled during their entire hospital stay. SFTSV RNA copies were investigated using real-time RT-PCR. The expression levels of apoptotic markers (annexin V and CD95) and proliferation and activation markers (Ki-67, HLA-DR, and CD25) and the expression levels of programmed cell death-1 (PD-1), interferon gamma (IFN-γ), and granzyme B in T cells were evaluated by flow cytometry for the SFTS patients.

Results: In parallel with T cell depletion, higher annexin V and CD95 expression was observed in SFTS patients. Additionally, the expression levels of Ki-67, HLA-DR, CD25, and PD-1 and the levels of IFN-γ and granzyme B in T lymphocytes were markedly increased in the SFTS patients.

Conclusions: T cell proliferation, activation, and functional enhancement were apparent despite the observation of T cell apoptosis, suggesting that these processes are involved in the complex protective response to SFTSV infection.

Introduction

Severe fever with thrombocytopenia syndrome (SFTS) is characterized by the sudden onset of high fever, leukocytopenia, thrombocytopenia, respiratory symptoms, gastrointestinal, or neurological symptoms and possibly even death within 7–14 days after disease onset, with a reported lethality rate varying from 12% to 30% (Yu et al., 2011; Wang et al., 2017). According to data released by the Centers for Disease Control (CDC) of China, SFTS has expanded to at least 19 provinces/municipalities in mainland China and has been reported in other countries, including Japan, South Korea, and the USA (Wang et al., 2017). Therefore, a better understanding of the mechanism by which the SFTS virus (SFTSV) causes illness is urgently needed. Additionally, defining human immune responses to SFTSV infection, disease pathogenesis, and protective variables is important for designing effective therapeutic and vaccination interventions.

Although knowledge on the mechanism underlying SFTS is incomplete, high viral RNA levels at admission and the overproduction of inflammatory cytokines have been reported to significantly contribute to the severity of SFTS (Wang et al., 2017; Gai et al., 2012; Sun et al., 2012). Additionally, innate immune cells, such as monocytes, natural killer cells, and myeloid and

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plasmacytoid dendritic cells (DCs), have been associated with different clinical outcomes (Zhang et al., 2017; Peng et al., 2016; Lu et al., 2015). A massive loss of T cells has also been observed in vivo during SFTSV infection in mice (Jin et al., 2012) and humans (Lu et al., 2015; Sun et al., 2014; Liu et al., 2017). This study aimed to further the knowledge acquired in previous studies and focused on the kinetics of the proliferation, activation, and functionality of T lymphocytes and the factors that may lead to T cell depletion in SFTS patients.

This study characterized the kinetics of T cell functionality and the expression of apoptotic/proliferation and activation/inhibition markers in T cells from 28 surviving SFTS patients soon after symptom onset and throughout the hospital stay. The data suggested that there was a possible involvement of apoptotic pathways (Fas/FasL interaction) in the loss of T lymphocytes during SFTSV infection. Additionally, T lymphocytes exhibited significantly higher proliferative activity, cytolytic activity, and effector functions in SFTSV-infected patients. This information will be useful in guiding more in-depth investigations into the mechanisms of SFTSV pathogenesis.

Materials and methods

Patients

To better characterize the kinetics of T cell characteristics, only the surviving SFTS patients admitted to the hospital by days 3–10 after disease onset were analyzed. A total of 28 patients who received standard antiviral and supportive treatments after admission to the hospital, based on SFTS treatment guidelines from the Chinese Ministry of Health, were identified. Individuals who were concurrently infected with hepatitis C virus (HCV), hepatitis B virus (HBV), or human immunodeficiency virus (HIV)-1, or who met the clinical or biological criteria for bacterial or fungal infections were excluded. A flow diagram of the enrolment process in this study is shown in the **Supplementary Material** Figure S1 in the online version at DOI: 10.1016/j.ijid.2018.03.010. The healthy controls were 15 uninfected blood donors who were matched to the infected patients by sex, age, and ethnic background. Patient details (including clinical history and routine hematological laboratory results) were collected from the medical records. The basic clinical and laboratory characteristics of the participants are listed in Tables 1 and 2.

Table 1
Differences in clinical and laboratory characteristics between SFTSV-infected patients and uninfected controls.

Index[a]	Uninfected controls ($n = 15$)	SFTS patients ($n = 28$)	p-Value[b]
Age, years	59 (27–66)	61 (35–74)	0.15[c]
Sex, male, n (%)	8 (53.3)	13 (46.4)	0.14[d]
Plasma RNA, $\log_{10}$	N/A	4 (1.13–6.95)	N/A
Platelet count, 10^9/l	160 (126–314)	8.5 (11–82)	<0.0001[c]
Monocyte count, 10^9/l	0.48 (0.34–0.60)	0.24 (0.01–1.44)	0.0011[c]
Lymphocyte count, 10^9/l	1.70 (1.33–2.08)	0.72 (0.21–2.31)	<0.0001[c]
Leukocyte count, 10^9/l	5.8 (4.22–9.45)	2.87 (0.75–12.62)	0.006[c]
ALT, U/l	20 (5–39)	66.5 (16–858)	<0.0001[c]
AST, U/l	19 (12–35)	164 (29–3876)	<0.0001[c]
CK, U/l	51 (13–119)	619 (77–2845)	<0.0001[c]
LDH, U/l	185 (139–218)	635 (268–6394)	<0.0001[c]

SFTSV, severe fever with thrombocytopenia syndrome virus; SFTS, severe fever with thrombocytopenia syndrome; N/A, not applicable; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase.
[a] All data except for the proportion of males are presented as the median (range).
[b] Statistically significant difference between the SFTS patients and the healthy controls; $p < 0.05$ was considered statistically significant.
[c] Based on the Mann–Whitney U-test.
[d] Based on the Pearson Chi-square test.

Sample collection and processing

Blood samples were collected from the patients at regular intervals (within 24 h of admission and every third day thereafter) during their entire hospital stay. Blood samples from uninfected donors were collected at the time of enrolment. All samples were processed within the first 4 h after collection. Peripheral blood mononuclear cells (PBMCs) were separated using density gradient centrifugation with Ficoll-Paque Plus (Dakewe Biotech, China) according to the manufacturer's instructions.

Antibodies and flow cytometry

A total of 2×10^5 PBMCs were collected in tubes and then stained with fluorescent conjugates of anti-human monoclonal antibodies (mAbs). Specifically, the CD3-APC-Cy7 (clone HIT3a), CD4-PerCP-cy5.5 (clone OKT4), CD8a-PE-Cy7 (clone HIT8a), CD95-PE (clone DX2), HLA-DR-PE-Cy7 (clone H243), CD25-PE (clone BC96), PD-1-PE-Cy7 (clone NKT105) mAbs and the FITC-Annexin V apoptosis detection kit (catalogue number 640914) were purchased from BioLegend (San Diego, CA, USA). The eFluor 506-labelled Fixable Viability Dye (catalogue number 65-0866-18) was purchased from eBioscience (Carlsbad, CA, USA). Isotype-matched control mAbs were used in all experiments. The PBMCs were incubated for 20 min at 4 °C with the indicated mAbs. After two washes with phosphate-buffered saline (PBS) (HyClone, USA), the samples were resuspended in 200 μl of PBS and run on a FACSCalibur Flow cytometer (BD Biosciences).

To measure intracellular cytokine (IFN-γ), approximately 2×10^5 PBMCs were stimulated with Phorbol-12-myristate-13-acetate (PMA) (50 ng/ml; Sigma-Aldrich, USA) and ionomycin (1 μg/ml; Sigma-Aldrich) in the presence of brefeldin A (Brefeldin A solution (BFA); 10 mg/ml; eBioscience) for 5 h of culture. For the analysis of cytotoxicity and proliferation markers, PBMCs were collected in tubes without PMA, BFA, or ionomycin stimulation. Subsequently, the PBMCs were stained with mAbs specific for membrane antigens, as described above, and permeabilized with Cytofix/Cytoperm (BD Biosciences, USA), followed by staining with IFN-γ (clone 4S.B3), granzyme B (clone QA16A02), and Ki-67 (clone Ki-67) antibodies (BioLegend) for 20 min at 4 °C. Finally, the cells were analyzed on a FACSCalibur Flow cytometer (BD Biosciences). A total of 5×10^4 to 1.0×10^5 events were acquired for each sample and analyzed using FlowJo software (Tree Star, Ashland, OR, USA).

SFTS viral load assay

As described previously (Zhang et al., 2017; Peng et al., 2016), total RNA was extracted from the patient serum samples using a viral RNA kit (DAAN Gene, Guangzhou, China), and the SFTSV RNA copies were evaluated using a certified real-time PCR kit (SFDA registration number 340166, China) following the manufacturer's instructions.

Statistical analysis

Unless otherwise indicated in the figure/table legends, the following statistical tests were used. For comparisons of two independent groups, the two-tailed Mann–Whitney U-test was performed. Statistical analyses were performed using GraphPad Prism 7.03 (GraphPad Software Inc., San Diego, CA, USA) and SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). The sample distribution was illustrated with box-and-whisker plots. In all figures, the horizontal bar indicates the median. The bars (whiskers) represent the sample distribution from the 10th percentile (lower bar) to the 90th percentile (upper bar). For all tests, the level of statistical

Table 2
Differences in the clinical and laboratory characteristics between different courses of the surviving SFTS patients and the uninfected controls.

Index[a]	Uninfected controls ($n = 15$)	SFTS patients ($n = 28$)				
		3–7 days ($n = 19$)	8–10 days ($n = 28$)	11–13 days ($n = 28$)	14–16 days ($n = 25$)	≥16 days ($n = 13$)
Platelet count, 10^9/l	160 (126–314)	49.5 (16–82)[b]****	107 (11–203)[b]****	149 (21–277)[b]***	198 (117–393)[b]*	201 (24–294)[b] ns
Monocyte count, 10^9/l	0.48 (0.34–0.60)	0.81 (0.01–1.09)[b]***	0.84 (0.01–1.46)[b]*	1.08 (0.11–1.12)[b]*	0.6 (0.21–1.22)[b]*	0.58 (0.22–0.99)[b] ns
Lymphocyte count, 10^9/l	1.70 (1.33–2.08)	1.17 (0.29–2.05)[b]****	1.27 (0.35–2.19)[b]**	2.38 (0.59–4.16)[b]*	1.63 (0.87–2.75)[b]*	1.41 (0.77–2.23)[b] ns
Leukocyte count, 10^9/l	5.8 (4.22–9.45)	5.28 (0.75–9.8)[b]***	7.04 (1.45–12.36)[b]**	11.8 (2–21.59)[b] ns	4.52 (2.36–7.33)[b] ns	4.42 (3.22–8.69)[b] ns
ALT, U/l	20 (5–39)	437 (16–858)[b]****	156 (22–290)[b]****	192.5 (27–558)[b]****	61.5 (22–831)[b]**	40 (27–60)[b]*
AST, U/l	19 (12–35)	1953 (29–3876)[b]****	718 (26–1410)[b]****	216.5 (23–410)[b]****	41 (20–263)[b]*	32 (19–58)[b]*
CK, U/l	51 (13–119)	6146 (94–12198)[b]****	1372.4 (21–2724)[b]****	308.5 (13–604)[b] ns	35.5 (12–67)[b] ns	68 (50–270)[b]*
LDH, U/l	185 (139–218)	3581 (268–6894)[b]****	1141 (174–2108)[b]***	717.5 (293–1142)[b]**	286 (204–382)[b]**	309.5 (201–552)[b]*

SFTS, severe fever with thrombocytopenia syndrome; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase. The level of statistical significance is indicated as follows: ns, not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.
[a] All data are presented as the median (range).
[b] Statistically significant difference between the SFTS patients and the healthy controls based on the Mann–Whitney *U*-test.

significance is indicated as follows: ns, not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

Results

Characterization of the uninfected controls and the SFTSV-infected patients

Twenty-eight surviving SFTS patients and 15 uninfected controls were enrolled in this study. Consistent with other reports (Gai et al., 2012; Zhang et al., 2017; Peng et al., 2016; Liu et al., 2014), the SFTS patients showed significantly lower total leukocyte, monocyte, lymphocyte, and platelet counts and higher serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), and lactate dehydrogenase (LDH) levels upon admission than the uninfected individuals. Additionally, as shown in Table 2 and **Supplementary Material** Figure S2 in the online version at DOI: 10.1016/j.ijid.2018.03.010, total lymphocyte and platelet counts were lower on days 3–7, continued to rise, and returned to nearly normal levels by day 16 after disease onset. Total leukocyte and monocyte counts were also lower on days 3–7, continued to rise, with a peak at days 11–13, and returned to nearly

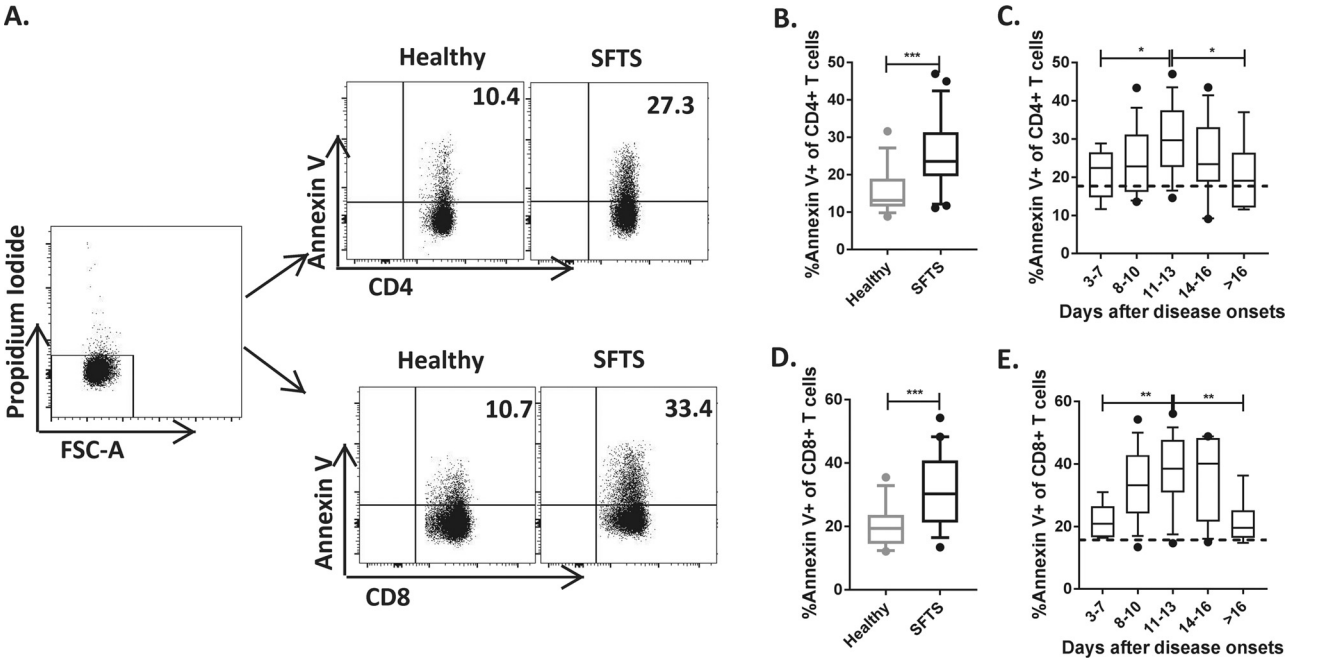

Figure 1. Apoptosis may lead to T lymphocyte loss during acute SFTSV infection. (A) The annexin V expression levels in CD4 and CD8 T cells from one representative uninfected control and one representative SFTS patient were analyzed by flow cytometry. (B) (D) The annexin V expression of CD4 and CD8 T cells is summarized for the uninfected controls ($n = 15$, grey boxes) and the SFTS patients upon admission ($n = 28$, black boxes). (C) (E) The dynamic changes in the annexin V expression of CD4 and CD8 T cells in the SFTS patients. These parameters were analyzed at different time intervals for the entire hospital stay. The dashed line represents the median of the uninfected controls. The ends of the whiskers in the box-and-whisker plots represent the 10th and 90th percentiles.

normal levels by day 16 after disease onset. Additionally, serum levels of ALT, AST, CK, and LDH were significantly higher on days 3–7, continued to decline, and returned to nearly normal levels by day 16 after disease onset. The results of the hematology laboratory tests for the uninfected individuals and the SFTS patients are shown in Tables 1 and 2.

Peripheral T lymphocyte apoptosis is probably partially mediated by Fas/FasL interactions

Twenty-eight surviving SFTSV-infected patients and 15 uninfected controls were included in the study. First, the absolute CD4 and CD8 T cell counts were determined. Significantly decreased absolute numbers of CD4 and CD8 T cells were observed during early SFTSV infection (**Supplementary Material** Figure S3 in the online version at DOI: 10.1016/j.ijid.2018.03.010). Subsequently, the expression of apoptotic markers (annexin V and CD95) in T lymphocytes was evaluated via flow cytometry to identify the mechanisms underlying the notable T lymphocyte death observed in **Supplementary Material** Figure S3 in the online version at DOI: 10.1016/j.ijid.2018.03.010. The data showed that CD4 and CD8 T cells from the SFTS patients expressed significantly higher annexin V and CD95 levels upon admission than those from the uninfected controls (Figure 1B, D and Figure 2C, E; $p < 0.01$ for both comparisons).

The dynamic data showed that the frequency of annexin V-expressing T cells continued to rise, with a peak at days 11–13 followed by a decline and a return to nearly normal levels by day 16 after disease onset (Figure 1C, E). Additionally, the expression of CD95+ T cells in the SFTS patients consistently fluctuated above normal values during the entire course and did not return to normal by day 16 after disease onset (Figure 2D, F).

Dynamic changes in T cell proliferation in the acute SFTSV-infected patients

To determine the changes in the proliferation of T cells, the expression of Ki-67 in CD4 and CD8 T cells was evaluated via flow cytometry in all 28 SFTS patients during their hospital stay. The percentages and counts of CD4 + Ki-67+ and CD8 + Ki-67+ T cells were significantly higher in the SFTSV-infected patients upon admission than in the uninfected controls (Figure 3C, E; $p < 0.01$ for all comparisons). In addition, the dynamic data showed that Ki-67 expression levels increased continuously, with a peak at days 11–13 in CD4 T cells and at days 8–10 in CD8 T cells, with a decline thereafter; however, the levels did not return to normal by day 16 after symptom onset (left panels of Figure 3D, F). Moreover, the kinetics of the proportion of Ki-67-expressing T cells were consistent with the relative absolute numbers (right panels of Figure 3D, F).

Dynamic changes in T cell activation during acute SFTSV infection

In accordance with the methods described in previous studies (Lindgren et al., 2011; McElroy et al., 2015; Rubin et al., 1985; Agrati et al., 2016), HLA-DR and CD25 expression levels of CD4 and CD8 T

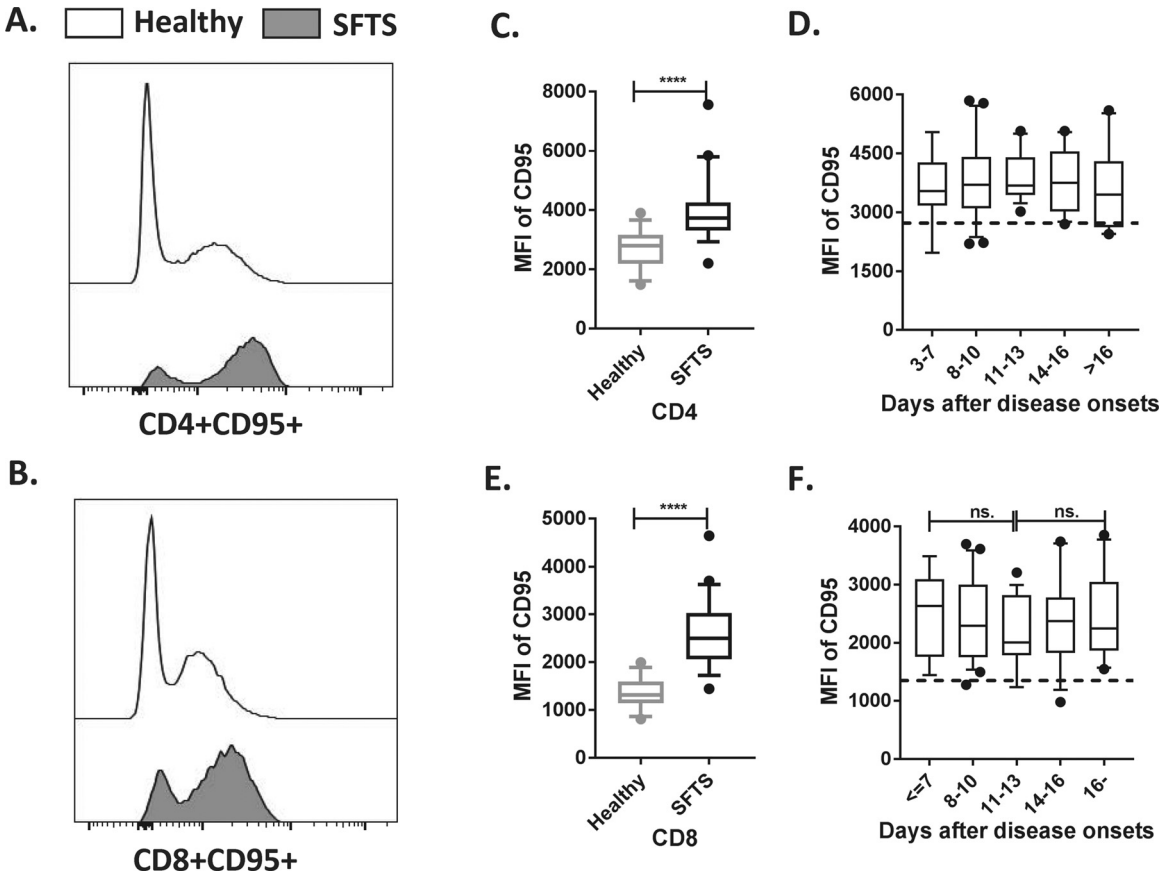

Figure 2. Peripheral T lymphocyte apoptosis is probably partially mediated by Fas/FasL interactions. (A) (B) The CD95 expression levels in CD4 and CD8 T cells from one representative uninfected control and one representative SFTS patient were analyzed by flow cytometry. (C) (E) The CD95 expression of CD4 and CD8 T cells was summarized for the uninfected controls ($n = 15$, grey boxes) and SFTS patients upon admission ($n = 28$, black boxes). (D) (F) Dynamic changes in the CD95 expression of CD4 and CD8 T cells in the SFTS patients. These parameters were analyzed at different time intervals for the entire hospital stay. The dashed line represents the median of the uninfected controls. The ends of the whiskers in the box-and-whisker plots represent the 10th and 90th percentiles.

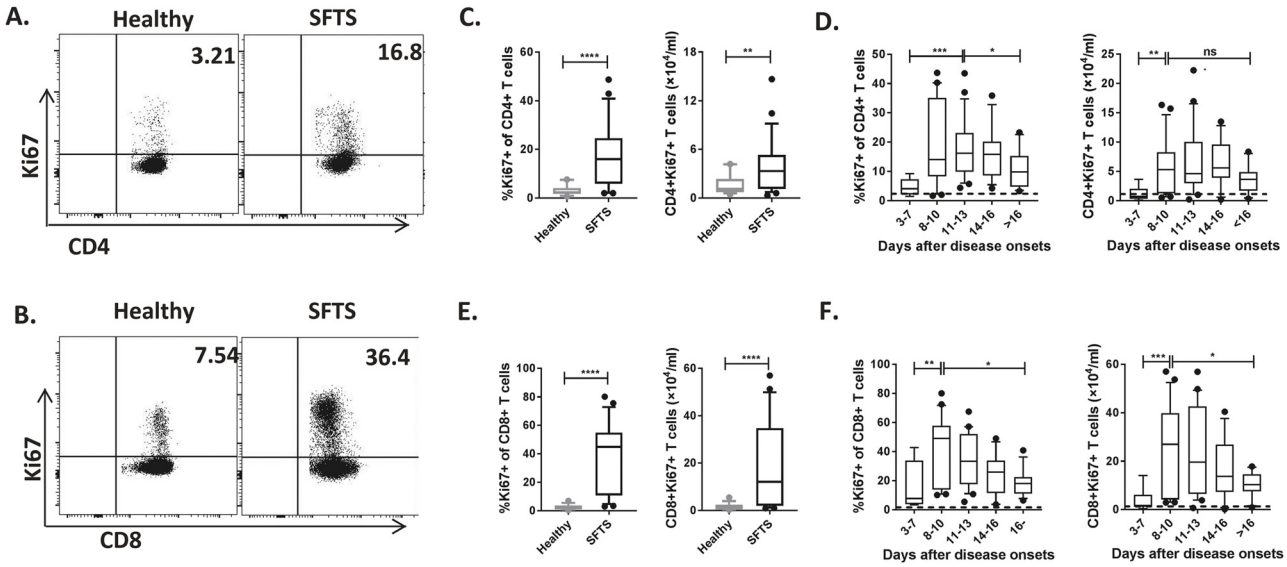

Figure 3. Dynamic changes in T cell proliferation in the acute SFTSV-infected patients. (A) (B) The Ki-67 expression levels in CD4 and CD8 T cells from one representative uninfected control and one representative SFTS patient were analyzed by flow cytometry. (C) (E) The percentages and absolute numbers of CD4 + Ki-67+ (C) and CD8 + Ki-67+ (E) T cells in the uninfected controls ($n = 15$, grey boxes) and SFTS patients upon admission ($n = 28$, black boxes). (D) (F) Dynamic changes in the percentages and absolute numbers of CD4 + Ki-67+ (D) and CD8 + Ki-67+ (F) T cells in the SFTS patients. These parameters were analyzed at different time intervals for the entire hospital stay. The dashed line represents the median of the uninfected controls. The ends of the whiskers in the box-and-whisker plots represent the 10th and 90th percentiles.

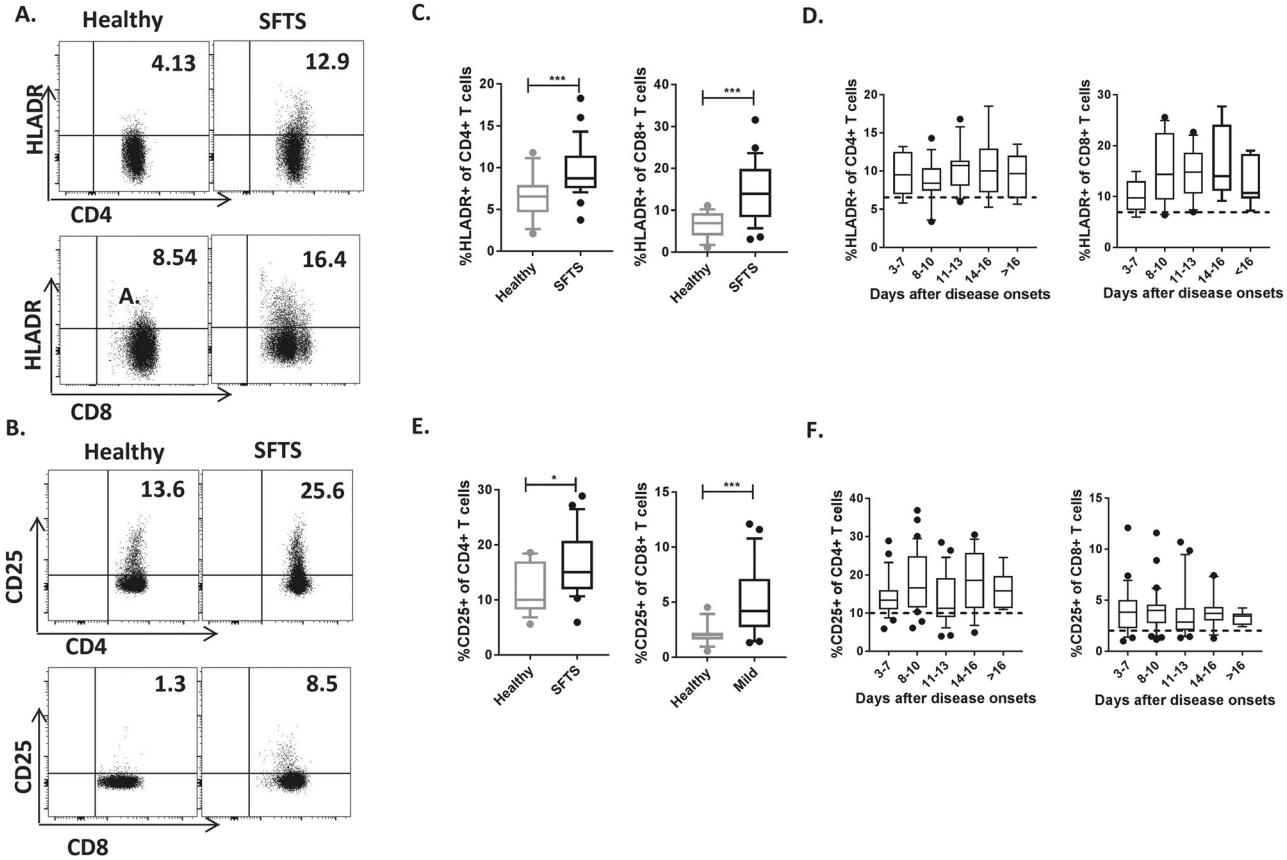

Figure 4. Dynamic changes in T cell activation during acute SFTSV infection. (A) (B) The HLA-DR (A) and CD25 (B) expression levels of CD4 and CD8 T cells from one representative uninfected control and one representative SFTS patient were analyzed by flow cytometry. (C) (E) The HLA-DR (C) and CD25 (E) expression levels in CD4 and CD8 T cells were summarized for the uninfected controls ($n = 15$, grey boxes) and the SFTSV-infected patients upon admission ($n = 28$, black boxes). (D) (F) Dynamic changes in the HLA-DR (D) and CD25 (F) expression on CD4 and CD8 T cells in the SFTS patients. These parameters were analyzed at different time intervals for the entire hospital stay. The dashed line represents the median of the uninfected controls. The ends of the whiskers in the box-and-whisker plots represent the 10th and 90th percentiles.

cells were evaluated to assess T cell activation via flow cytometry in all 28 SFTS patients during their hospital stay. The HLA-DR and CD25 expression levels in CD4 and CD8 T cells were significantly higher in the SFTSV-infected patients upon admission than in the uninfected controls (Figure 4C, E; $p < 0.05$ for all comparisons).

Additionally, HLA-DR expression of the CD4 and CD8 T cells peaked at days 11–13 and did not return to normal levels by day 16 (Figure 4D). Furthermore, the CD25 expression kinetics of CD4 T cells paralleled those in CD8 T cells, fluctuating above normal levels, deceasing to the lowest levels on days 11–13, and then increasing without returning to normal levels by day 16 after symptom onset (Figure 4F).

Kinetics of programmed cell death-1 (PD-1) expression of CD4 and CD8 T cells during acute SFTSV infection

PD-1, an inhibitory receptor in the B7-CD28 family, has been linked to T cell dysfunction in settings of chronic viral infection (Keir et al., 2007), but this association has not yet been evaluated during acute SFTSV infection in humans. To address this issue, PD-1 expression of T cells in SFTS patients was evaluated in the present study. The data showed that PD-1 expression of CD4 and CD8 T cells was significantly higher in the SFTSV-infected patients upon admission than in the uninfected controls (Figure 5C, E; $p < 0.0001$ for all comparisons). Additionally, PD-1 expression of CD4 and CD8 T cells continued to rise, with a peak at days 11–13 followed by a decline and a return to nearly normal by approximately day 16 (Figure 5D, F).

Dynamic changes in T cell effector and cytotoxicity functions in SFTS patients

To determine the kinetics of the T cell functionality, IFN-γ (Figure 6D–G) and granzyme B (Figure 6H, I) expression was evaluated dynamically in all 28 SFTS patients via flow cytometry. The data showed that the CD4 and CD8 T cells expressed significantly higher levels of IFN-γ in the SFTS patients upon admission than in the uninfected controls (Figure 6D, F; $p < 0.0001$ for both comparisons). In addition, granzyme B expression levels in CD8 T cells were higher among SFTS patients upon admission than among uninfected controls (Figure 6H, $p < 0.001$).

The dynamic data showed that the frequency of IFN-γ-expressing T cells continued to rise, with a peak at days 11–13 followed by a decline and a return to normal by approximately day 16 after disease onset (Figure 6E, G). Additionally, the expression levels of CD8+ granzyme B+ T cells continued to rise, with a peak at days 8–11 followed by a decline and a return to normal levels around days 14–16 (Figure 6I).

Discussion

Defining the immunological responses to SFTSV infection in humans represents a key issue in identifying a protective profile that can be useful for pathogenesis studies and vaccine development. To explore the primary human T cell responses to acute SFTSV infection, a cohort of surviving SFTS patients was followed throughout the entire hospital stay. The results of this study showed that the critical laboratory parameters returned to nearly normal levels by day 16 after disease onset in the surviving SFTS patients. Moreover, peripheral T lymphocyte apoptosis was probably partially mediated by Fas/FasL interactions. Additionally, Ki-67, HLA-DR, CD25, and PD-1 expression and the levels of granzyme B and IFN-γ in T cells were markedly increased in the SFTSV-infected patients. Although the SFTS patients in this study developed marked lymphopenia, a significant proportion of their lymphocytes was activated and showed functional enhancement, suggesting that a potent adaptive immune response to the SFTSV was initiated in these patients.

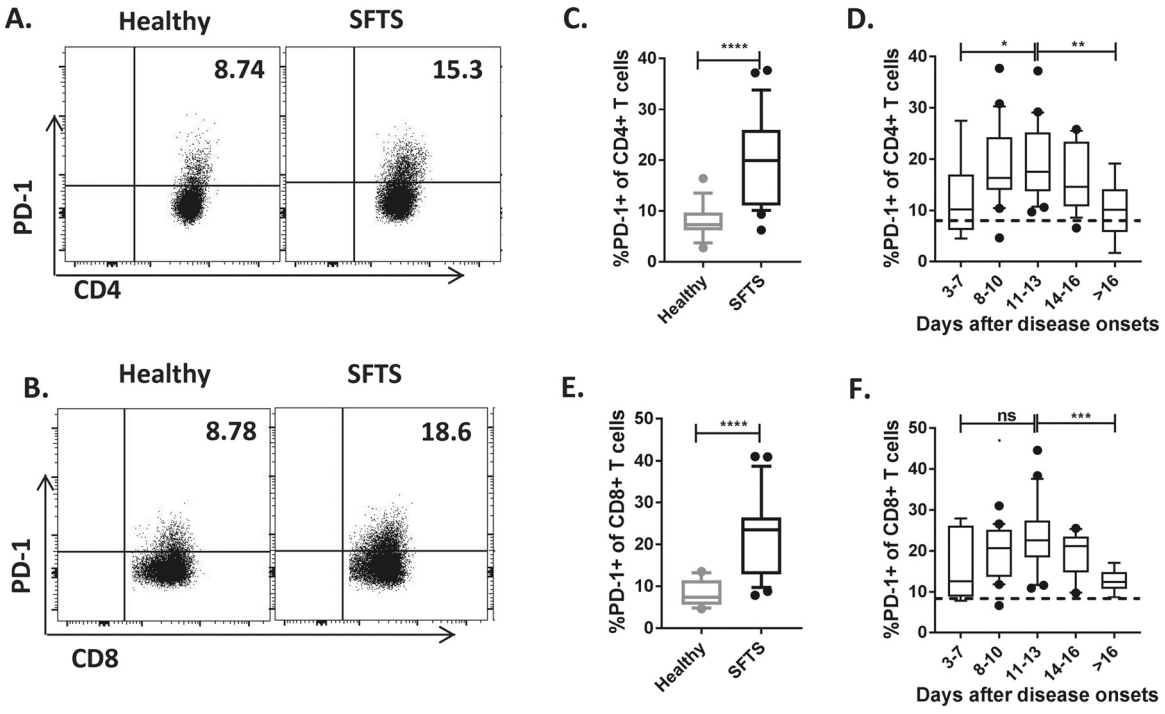

Figure 5. Kinetics of the PD-1 expression of CD4 and CD8 T cells during acute SFTSV infection. (A) (B) The PD-1 expression levels of CD4 and CD8 T cells from one representative healthy control and one representative SFTS patient were analyzed by flow cytometry. (C) (E) The PD-1 expression of CD4 and CD8 T cells was summarized for the uninfected controls ($n = 15$, grey boxes) and the SFTS patients upon admission ($n = 28$, black boxes). (D) (F) Dynamic changes in the PD-1 expression of CD4 and CD8 T cells in the SFTS patients. These parameters were analyzed at different time intervals for the entire hospital stay. The dashed line represents the median of the uninfected controls. The ends of the whiskers in the box-and-whisker plots represent the 10th and 90th percentiles.

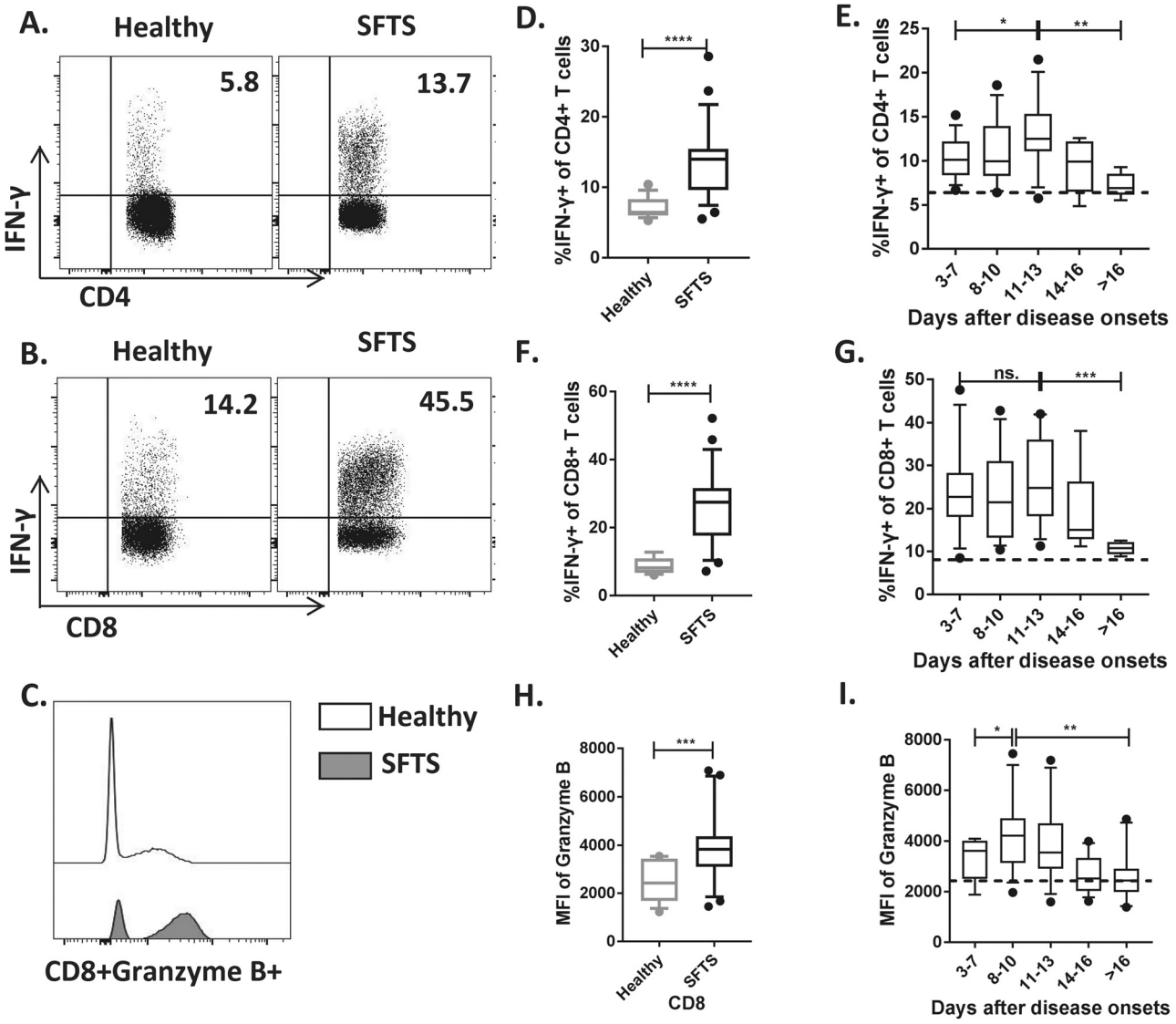

Figure 6. Dynamic changes in T cell effector and cytotoxicity functions in the SFTS patients. (A) (B) (C) Representative examples of IFN-γ (A and B) and granzyme B (C) expression of CD4 and CD8 T cells in one uninfected control and one SFTS patient were analyzed by flow cytometry. (D) (F) (H) IFN-γ (D and F) and granzyme B (H) expression of CD4 and CD8 T cells was summarized for the uninfected controls ($n = 15$, grey boxes) and the SFTS patients upon admission ($n = 28$, black boxes). (E) (G) (I) Dynamic changes in the IFN-γ (E and G) and granzyme B (I) expression of CD4 or CD8 T cells in the SFTS patients. These parameters were analyzed at different time intervals for the entire hospital stay. The dashed line represents the median of the uninfected controls. The ends of the whiskers in the box-and-whisker plots represent the 10th and 90th percentiles.

In line with previous studies (Lu et al., 2015; Sun et al., 2014; Liu et al., 2017), the SFTS patients showed significantly lower leukocyte, monocyte, lymphocyte, and platelet counts and higher serum levels of ALT, AST, CK, and LDH upon admission than the uninfected individuals, and these measurements returned to nearly normal levels by day 16 after disease onset. Additionally, previous studies (Lu et al., 2015; Sun et al., 2014; Liu et al., 2017) have suggested that T cell deficiency is commonly observed during the early course of SFTSV infection, especially in patients with severe disease. This study expanded on previous studies and found that the dynamic changes in T lymphocytes (including CD4 + T cells and CD8 + T cells) were consistent with the kinetics of the lymphocytes and returned to nearly normal levels by approximately day 16 after the onset of fever.

As is already known, the loss of T cells in SFTS patients may affect the direct antiviral functions of the cells that regulate immunopathology and mediate the cytotoxic killing of virus-infected cells. However, no study has reported the mechanisms underlying T lymphocyte loss in SFTS patients. Apoptosis is the most extensively investigated programmed cell death pathway during viral infection (Danthi, 2016). Previous reports concerning the Ebola virus have suggested that Ebola virus infection of monocytes and DCs can result in the induction of the Fas (also called CD95) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) pathways, which may contribute to T lymphocyte apoptosis (Hensley et al., 2002; Iampietro et al., 2017; Yang et al., 2001; Wauquier et al., 2010; Falasca et al., 2015). Similar to the findings of previous studies on Ebola virus (Wauquier et al., 2010; Falasca et al., 2015), the present study data suggested that the annexin V and CD95 expression levels were significantly higher in CD4 and CD8 T cells from the SFTS patients than in those from the uninfected controls; this suggests that T cell apoptosis might occur through the Fas/FasL pathway. However, further investigation is required to elucidate other apoptotic pathways and determine the molecular mechanisms leading to T cell apoptosis in SFTS patients.

Analysis of the Ki-67, HLA-DR, and CD25 expression patterns in CD4 and CD8 T cells at multiple time points allowed the evaluation of the magnitude and kinetics of effector T cell responses after acute SFTSV infection. Increased Ki-67 expression of T cells from SFTS patients suggested that the gradual increase in T cell numbers was a direct consequence of induced proliferation (Gerdes et al., 1984). Additionally, T cell proliferation and activation might be associated with increased expression of apoptotic markers, as has been suggested by studies on Ebola virus infection (McElroy et al., 2015; Agrati et al., 2016; Falasca et al., 2015). In terms of time and magnitude, T cell activation early after acute SFTSV infection in the present study matched or was even earlier than that after Ebola virus infection but later than that after hantavirus infection (Lindgren et al., 2011; McElroy et al., 2015; Agrati et al., 2016). Nevertheless, T cell responses to chronic infections (e.g., HBV and HCV) can take much longer to develop after infection (Thimme et al., 2002; Thimme et al., 2003). The present study data may suggest that a rapid response to SFTSV infection represents the optimal response, whereas slower responses are suboptimal.

Previous studies have indicated that PD-1 is highly expressed in dysfunctional CD8 and CD4 T cells during chronic infections and contributes to the dampening of antiviral T cell immunity (Keir et al., 2007). The precise role of PD-1 during acute viral infections has not been defined. A previous study concerning the rabies virus suggested that the PD-1–PD-L pathway can limit the damage caused by over-aggressive T cells (Iwai et al., 2003; Lafon et al., 2008). Other studies have suggested that PD-1 may play a regulatory role during acute infection, as its expression is rapidly up-regulated upon T cell activation, and thus may also serve as a marker of T cell activation (Araki et al., 2013; Kosinska et al., 2017). PD-1 expression of CD4 and CD8 T cells was evaluated early after acute SFTSV infection and it was found that nearly 20% of the CD4 and CD8 T cells exhibited signs of PD-1 expression early after SFTSV infection, which is similar to human Ebola virus infection (Agrati et al., 2016). Moreover, the kinetics of the proportion of PD-1-expressing T cells were consistent with the kinetics of T cell activation markers (Ki67 and HLA-DR). Increased PD-1 expression of CD4 and CD8 T cells suggests that CD4 and CD8 T cells may regulate the strength of the effector responses via the same pathways during acute SFTSV infection.

In parallel with T cell activation and inhibition, increased cytotoxic functions (granzyme B) of CD8 T cells and increased effector functions (IFN-γ) of CD4 and CD8T cells were observed in SFTS patients during the acute phase, which was similar to observations from studies on other acute viral infections (Lindgren et al., 2011; McElroy et al., 2015; Agrati et al., 2016). In terms of time and magnitude, the levels of effector T cell responses observed in the present study matched or even exceeded those reported by earlier human vaccination studies (Miller et al., 2008). Moreover, consistent with the antigen-specific T cell responses against acute viral infections, CD4 T cell responses were lower in magnitude than CD8 T cell responses (McElroy et al., 2015; Miller et al., 2008). In summary, the present study data suggest that the CD4 and CD8 T cell responses occur very rapidly and vigorously in acute SFTSV infection. However, further research is required to determine whether the kinetics of T cell proliferation, activation, and functionality in SFTS patients are associated with changes in the viral load or in the viral antigens.

In conclusion, a potential pathway that mediates peripheral T lymphocyte apoptosis was revealed in this study, and the CD4 and CD8 T cell responses to acute SFTSV infection in humans were characterized. The study results indicate that the Fas/FasL pathway might contribute to T lymphocyte apoptosis in SFTS patients and that the effector responses were induced rapidly and vigorously after symptom onset. Additionally, inhibitory immunoregulatory components may actively modulate the effector responses. These data shed light on the development of this disease and contribute to a better understanding of the mechanism underlying the progression of SFTS.

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