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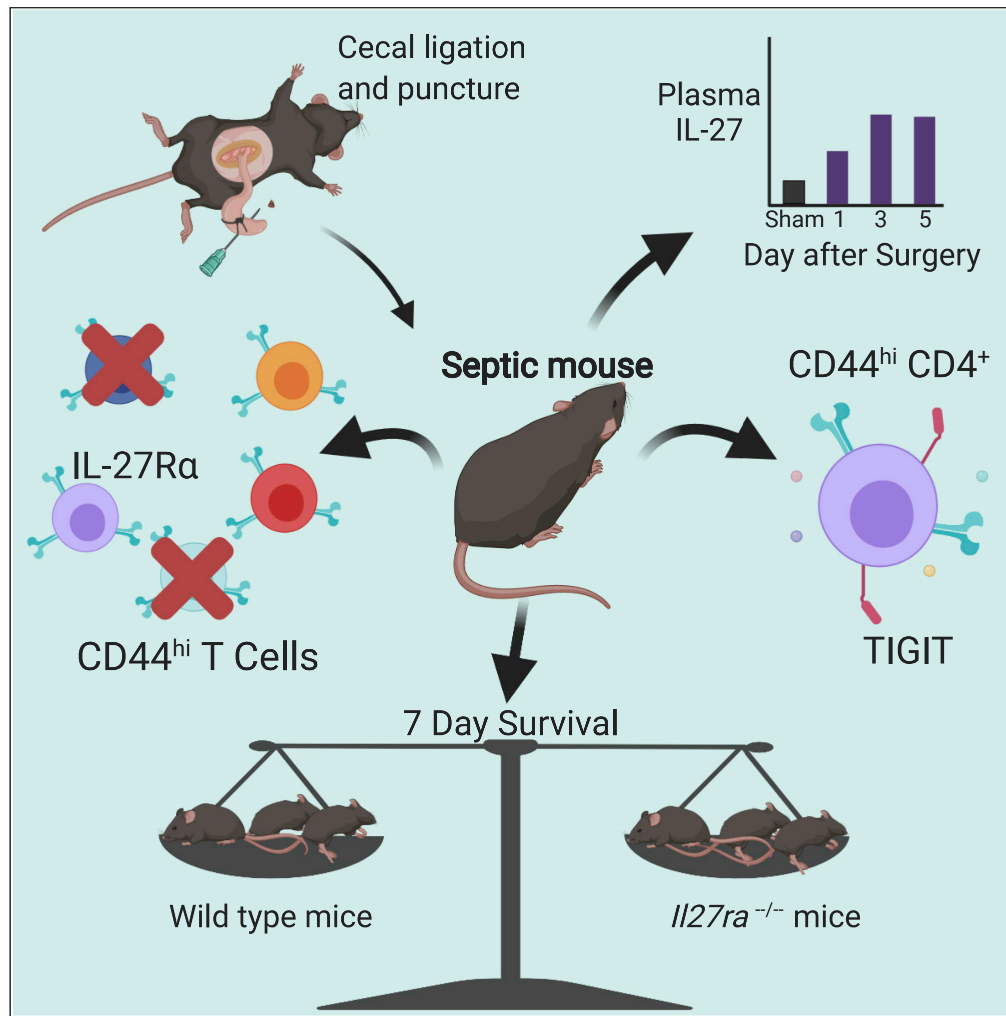

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HIGHLIGHTS

Numbers of IL-27Rα+ memory T cells are decreased following cecal ligation and puncture

TIGIT is expressed on more IL-27Rα+ versus IL-27Rα− memory CD4+ T cells during sepsis

Il27ra−/− and WT T cells exhibit similar effector function and apoptosis during sepsis

IL-27 signaling does not impact sepsis mortality

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The IL-27 receptor regulates TIGIT on memory CD4$^+$ T cells during sepsis

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SUMMARY

Sepsis is a leading cause of morbidity and mortality associated with significant impairment in memory T cells. These changes include the upregulation of co-inhibitory markers, a decrease in functionality, and an increase in apoptosis. Due to recent studies describing IL-27 regulation of TIGIT and PD-1, we assessed whether IL-27 impacts these co-inhibitory molecules in sepsis. Based on these data, we hypothesized that IL-27 was responsible for T cell dysfunction during sepsis. Using the cecal ligation and puncture (CLP) sepsis model, we found that IL-27Rα was associated with the upregulation of TIGIT on memory CD4$^+$ T cells following CLP. However, IL-27 was not associated with sepsis mortality.

INTRODUCTION

Sepsis is defined as a life-threatening organ dysfunction resulting from the body's dysregulated response to infection (Singer et al., 2016). It is a significant source of morbidity and mortality worldwide—in 2017 alone, an estimated 11 million people died from sepsis, accounting for 20% of all deaths that year (Rudd et al., 2020). Rapid recognition of sepsis and initiation of supportive care is effective at reducing mortality in the early stages of sepsis (Ferrer et al., 2009; Seymour et al., 2017), but a substantial proportion of sepsis patients die between 31 days and 2 years (Prescott et al., 2016). Mortality is associated with long-term immune system dysfunction that begins shortly after sepsis onset (Boomer et al., 2011).

Sepsis-induced immune dysfunction is multi-faceted, characterized by changes in both innate and adaptive cell responses. Current evidence suggests that alterations in the T cell repertoire lead to adaptive immune system dysfunction. We previously published data describing alterations in the T cell compartment following sepsis. We found significant reductions in the number of total CD4$^+$ and CD8$^+$ T cells using the cecal ligation and puncture (CLP) model of sepsis (Ramonell et al., 2017; Chen et al., 2017). Naive and memory CD4$^+$ T cell numbers are significantly reduced following CLP. Although the number of naive CD8$^+$ T cells is similar between CLP and sham-surgery mice at all time points, the number of memory CD8$^+$ T cells is reduced following sepsis (Serbanescu et al., 2016; Xie et al., 2019a). One day after CLP surgery, the number of memory CD8$^+$ T cells is reduced by 44% and remains reduced for three days after surgery (Serbanescu et al., 2016). Co-inhibitory markers such as TIGIT and PD-1 are also highly upregulated on T cells and linked to their apoptosis during sepsis (Boomer et al., 2012; Chen et al., 2017; Sjaastad et al., 2018; Ammer-Herrmenau et al., 2019; Xie et al., 2019a, 2019b).

Recently, the immunosuppressive cytokine IL-27 was associated with TIGIT and PD-1 expression on T cells during cancer (Chihara et al., 2018) and toxoplasmosis (Delong et al., 2019). Furthermore, IL-27 inhibited memory T cell responses during secondary malaria infection (Gwyer Findlay et al., 2014). We therefore hypothesized that IL-27 signaling is responsible for the upregulation of TIGIT and PD-1 on memory T cells during sepsis and ultimately for sepsis mortality. Previous studies also observed that the level of serum IL-27 increases following sepsis in mouse models and human septic patients (Wirtz et al., 2006; Nelson et al., 2010; Wong et al., 2012, 2013, 2014; Bosmann et al., 2014b; Cao et al., 2014; Hanna et al., 2015; Gao et al., 2016; Yan et al., 2016). Produced by antigen-presenting cells (Pflanz et al., 2002), IL-27 primarily regulates the effector response of dendritic cells and T cells during infections (Villarino et al., 2003; Yoshimura et al., 2006; Guzzo et al., 2012; Clement et al., 2016; Patin et al., 2016; Sowrirajan et al., 2017; Wehrens et al., 2018). Memory T cells express particularly high levels of the IL-27 receptor (IL-27Rα) (Villarino et al., 2005).

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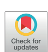

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To determine whether IL-27 signaling is responsible for co-inhibitory molecule expression on memory T cells during sepsis, we analyzed the phenotype and function of IL-27Rα$^+$ on memory T cells following cecal ligation and puncture (CLP). IL-27 receptor expression was associated with TIGIT, but not PD-1, expression on memory CD4$^+$ T cells, and impaired production of IFNγ. However, IL-27 signaling was not associated with sepsis mortality. These results suggest that IL-27Rα signaling upregulates TIGIT on memory CD4$^+$ T cells during sepsis but does not affect sepsis mortality.

RESULTS

Sepsis increases plasma IL-27 but is associated with a reduction in the frequency and number of IL-27Rα$^+$ CD4$^+$ and CD8$^+$ T cells

Although plasma IL-27 has been previously shown to increase at early time points during sepsis, it is not known how long it remains elevated (Wirtz et al., 2006; Nelson et al., 2010; Wong et al., 2012, 2013, 2014; Bosmann et al., 2014b; Cao et al., 2014; Hanna et al., 2015; Gao et al., 2016; Yan et al., 2016). To address this, mice underwent cecal ligation and puncture (CLP) or sham surgery to induce sepsis (see Transparent Methods). Plasma was collected on days 1 through 5 days following surgery. Septic animals had increased plasma IL-27p28 on days 1, 3, and 5 compared with mice that underwent sham surgery (Figure 1A). Although systemic IL-27p28 increased following CLP, it was unclear what proportion of T cells expressed the IL-27 receptor (IL-27Rα). Multi-color flow cytometry was used to determine the frequency and number of IL-27Rα expressing T cells in the spleens of CLP and sham mice. A high frequency of CD4$^+$ and CD8$^+$ T cells expressed IL-27Rα in unmanipulated mice (Figure S1). After CLP surgery, the frequency of IL-27Rα$^+$ cells among CD44^{lo} naive CD4$^+$ T cells was reduced compared with sham controls on days 2 and 4 (Figures 1B and 1C). In contrast, the frequency of IL-27Rα$^+$ CD44^{hi} memory CD4$^+$ T cells decreased on day 2 following CLP but increased back to sham levels on days 3 and 4 (Figures 1B and 1C). Because sepsis causes lymphopenia, we also assessed the absolute cell numbers. We found that absolute numbers of IL-27Rα$^+$ CD44^{lo} and CD44^{hi} CD4$^+$ T cells were significantly reduced after CLP, with a greater reduction in the CD44^{hi} population (Figure 1D). The frequency of IL-27Rα$^+$ CD44^{lo} or CD44^{hi} CD8$^+$ T cells was not altered after CLP (Figures 1E and 1F). However, the absolute number of IL-27Rα$^+$ CD44^{hi} (but not CD44^{lo}) CD8$^+$ T cells was reduced on days 2 and 3 after CLP (Figure 1G). Moreover, neither the number of IL-27Rα$^-$ CD44^{hi} nor IL-27Rα$^-$ CD44^{lo} T cells was not decreased following CLP (Figure S2).

IL-27 signaling is associated with the upregulation of TIGIT on memory CD4$^+$ T cells in septic mice

Based on the literature showing that IL-27 signaling regulates the expression of TIGIT and PD-1 (Delong et al., 2019; Chihara et al., 2018), we sought to determine whether IL-27Rα expression associates with the frequency of TIGIT and PD-1 expression on memory T cells during sepsis. To do this, we assessed the frequency of the co-inhibitory molecules TIGIT and PD-1 (gating strategy shown in Figure S2) in IL-27Rα$^+$ and IL-27Rα$^-$ CD44^{hi} CD4$^+$ T cells on days 1–4 after CLP. Beginning on day 3 and continuing to day 4 following CLP, the frequency of TIGIT$^+$ cells in the IL-27Rα$^+$ CD4$^+$ memory population increased relative to the IL-27Rα$^-$ CD4$^+$ memory population (Figures 2A and 2B). When comparing sham mice with CLP mice on days 1, 2, 3, and 4 following surgery, TIGIT expression was increased on IL-27Rα$^+$ memory CD4 T cells ($p = 0.01$), with $p = 0.004$ when comparing sham versus day 3 post-CLP and $p = 0.04$ when comparing sham versus day 4 post-CLP (Figures 2A and 2B). When making the same comparisons for IL-27Rα$^-$ memory CD4$^+$ T cells, the overall difference was not significant ($p = 0.37$) and $p > 0.99$ on days 3 and 4 after CLP. This suggests that sepsis increases the expression of TIGIT selectively on the IL-27Rα$^+$ cell population (Figures 2A and 2B). However, we found no difference in the frequency of PD-1$^+$ cells between IL-27Rα$^+$ versus IL-27Rα$^-$ memory CD4$^+$ T cell populations (Figures 2A and 2C). There were no significant differences in the frequencies of TIGIT$^+$ (Figures 2D and 2E) or PD-1$^+$ (Figures 2D and 2F) T cells at any point following CLP. In contrast to the memory CD4$^+$ T cell compartment, memory CD8$^+$ T cells had an indistinct population of TIGIT$^+$ and PD-1$^+$ cells (Figure 2E). These results demonstrate that IL-27 receptor expression is associated with TIGIT expression on CD44^{hi} CD4$^+$ T cells.

IL-27 signaling is associated with reduced proliferation, but not apoptosis, of CD44^{hi} CD8$^+$ T cells in septic mice

To determine whether IL-27 modulates the apoptosis or proliferation of T cells during sepsis, we next assessed whether the IL-27 signaling was associated with activity of the apoptotic proteases Caspase 3 and Caspase 7 or expression of the proliferation marker Ki67. We measured active Caspase 3/7 on memory

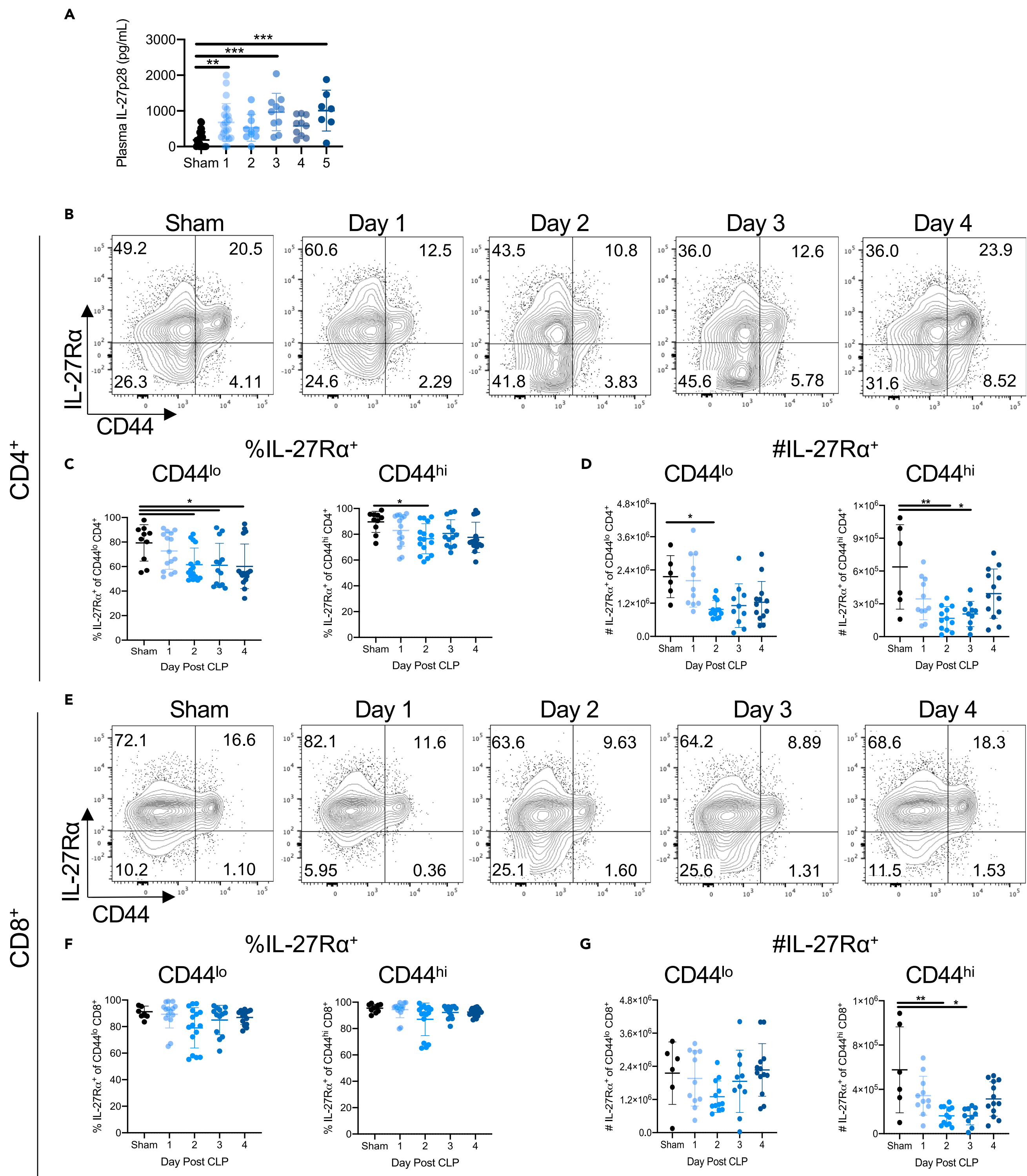


Figure 1. Sepsis results in a reduction in the frequency and number of IL-27Rα$^+$ T cells

Following cecal ligation and puncture (CLP) or sham surgery (sham), animals were euthanized on the indicated days. Plasma was collected for IL-27p28 ELISAs on days 1 through 5 and spleens harvested for analysis by flow cytometry on days 1 through 4.

(A) Concentration of IL-27p28 in the plasma of sham and CLP mice on days 1 through 5 following surgery.

(B) Representative flow cytometric plots showing the frequency of IL-27Rα expressing CD4$^+$ T cells in sham and CLP mice on days 1–4 after surgery.

(C) The frequency of CD4$^+$ CD44^{lo} naive (left) and CD4$^+$ CD44^{hi} memory (right) T cells expressing the IL-27Rα in sham and CLP mice on days 1–4 after surgery.

Figure 1. *Continued*

(D) The absolute number of CD4$^+$ CD44^{lo} naive (left) and CD4$^+$ CD44^{hi} memory (right) T cells expressing the IL-27Rα in sham and CLP mice on days 1–4 after surgery.

(E) Representative flow cytometric plots showing the frequency of IL-27Rα expressing CD8$^+$ T cells in sham and CLP mice on days 1–4 after surgery.

(F) The frequency of CD8$^+$ CD44^{lo} naive (left) and CD8$^+$ CD44^{hi} memory (right) T cells expressing the IL-27Rα in sham and CLP mice on days 1–4 after surgery.

(G) The absolute number of CD8$^+$ CD44^{lo} naive (left) and CD8$^+$ CD44^{hi} memory (right) T cells expressing the IL-27Rα in sham and CLP mice on days 1–4 after surgery. All summary data were pooled from three independent experiments, with n = 7–18 mice per group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Error bars represent the mean ± the standard deviation.

T cells using flow cytometry and found that CD44^{hi} CD4$^+$ T cells exhibit similar frequencies of active caspase 3/7$^+$ cells in both wild-type and *Il27ra*$^{-/-}$ mice (Figure 3A). Also, the frequency of caspase 3/7$^+$ apoptotic cells is similar between the CD44^{hi} CD8$^+$ T cells in wild-type and *Il27ra*$^{-/-}$ septic mice (Figure 3B). The frequency of proliferating (Ki67$^+$) cells among CD4$^+$ CD44^{hi} memory T cells was similar in IL-27Rα$^-$ versus IL-27Rα$^+$ populations (Figures 4A and 4B). We next looked at the association between TIGIT expression and proliferation in the IL-27Rα$^-$ and IL-27Rα$^+$ populations. Among IL-27Rα$^-$ memory CD4$^+$ T cells, TIGIT$^+$ cells exhibited reduced frequencies of proliferating cells compared with TIGIT$^-$ cells in non-septic animals but similar frequencies following CLP (Figure 4C). In contrast, TIGIT$^+$ cells proliferated more than TIGIT$^-$ IL-27Rα$^+$ memory CD4$^+$ T cells one day following CLP (Figure 4C). In the CD44^{hi} memory CD8$^+$ T cell compartment, IL-27Rα expression associated with reduced proliferation 1 and 2 days after CLP (Figures 4D and 4E). Among IL-27Rα$^-$ cells, there was no difference in proliferation based on TIGIT expression at any time point analyzed. In contrast, among IL-27Rα$^+$ T cells, TIGIT$^+$ cells proliferated more than TIGIT$^-$ cells one day after CLP surgery (Figure 4F). These results indicate that the numerical reduction in IL-27Rα$^+$ memory CD4$^+$ T cells following CLP is unrelated to a deficit in proliferation but that reduced proliferation may be responsible for the reduced numbers in the CD8$^+$ T cell compartment. In addition, TIGIT expression is associated with higher proliferation in IL-27Rα$^+$, but not IL-27Rα$^-$, memory T cells after CLP.

Septic mice lacking the IL-27Rα have a reduced frequency of TIGIT$^+$ memory CD4$^+$ T cells but a similar frequency of Treg compared with wild-type mice

After identifying an association between IL-27Rα positivity and TIGIT expression on CD4$^+$ memory T cells, we assessed TIGIT and PD-1 expression on memory T cells in *Il27ra*$^{-/-}$ mice to determine whether IL27Rα deficiency altered their expression. First, we compared the frequency of the co-inhibitory molecules TIGIT and PD-1 in the CD44^{hi} T cell compartment of *Il27ra*$^{-/-}$ with wild-type (WT) mice. We found that frequencies of TIGIT$^+$ cells among the memory CD4$^+$ T cell population increased following CLP in both WT and *Il27ra*$^{-/-}$ mice compared with the sham group (Figure 5A). However, on days 1 and 2 after CLP, *Il27ra*$^{-/-}$ mice had a significantly reduced frequency of TIGIT$^+$ memory CD4$^+$ T cells compared with WT mice (Figure 5A). We found that the frequency of PD-1$^+$ cells among CD44^{hi} CD4$^+$ T cells increased in both WT and *Il27ra*$^{-/-}$ mice following CLP compared with the sham group; however, there was no difference between WT versus *Il27ra*$^{-/-}$ mice in the sham or CLP groups (Figure 5B). In contrast to the memory CD4$^+$ T cell compartment, the memory CD8$^+$ T cell compartment of the WT versus *Il27ra*$^{-/-}$ mice showed no difference in the frequency of TIGIT$^+$ cells (Figure 5C) or PD-1$^+$ cells (Figure 5D) among CD44^{hi} CD8$^+$ T cells. These results further support the idea that IL-27 signaling enhances TIGIT expression on CD4$^+$ memory T cells.

Previous studies showed that IL-27 promotes the development and effector function of T regulatory cells (Tregs) (Do et al., 2017; Kim et al., 2019; Moon et al., 2013; Nguyen et al., 2019; Wehrens et al., 2018). Because many Tregs express the co-inhibitory receptor TIGIT, we next looked to determine the frequency of Tregs in wild-type and *Il27ra*$^{-/-}$ mice. The frequency of FoxP3$^+$ Tregs significantly increased in both wild-type and *Il27ra*$^{-/-}$ mice following CLP, but there was no difference in Treg frequency between these groups (Figures 6A and 6B). Within the TIGIT$^+$ CD4$^+$ T cell population, *Il27ra*$^{-/-}$ mice had a higher frequency of FoxP3$^+$ expression following CLP compared with mice that underwent sham surgery (Figure 6C). However, the frequency of Tregs within the TIGIT$^+$ population was unchanged in wild-type septic mice (Figure 6C).

Memory CD4$^+$ T cells in Il27ra$^{-/-}$ mice exhibit impaired production of IFN-γ, but this is not linked to TIGIT expression

To assess whether the IL-27-driven signals during sepsis impair the effector cytokine production, we measured IFN-γ and TNFα production by memory CD4$^+$ and CD8$^+$ T cells in wild-type compared with *Il27ra*$^{-/-}$ mice following CLP-induced sepsis. *Il27ra*$^{-/-}$ mice exhibited a reduced frequency of IFN-γ-producing CD44^{hi} CD4$^+$ T cells compared with WT in mice receiving sham surgery and day 1 after CLP-induced

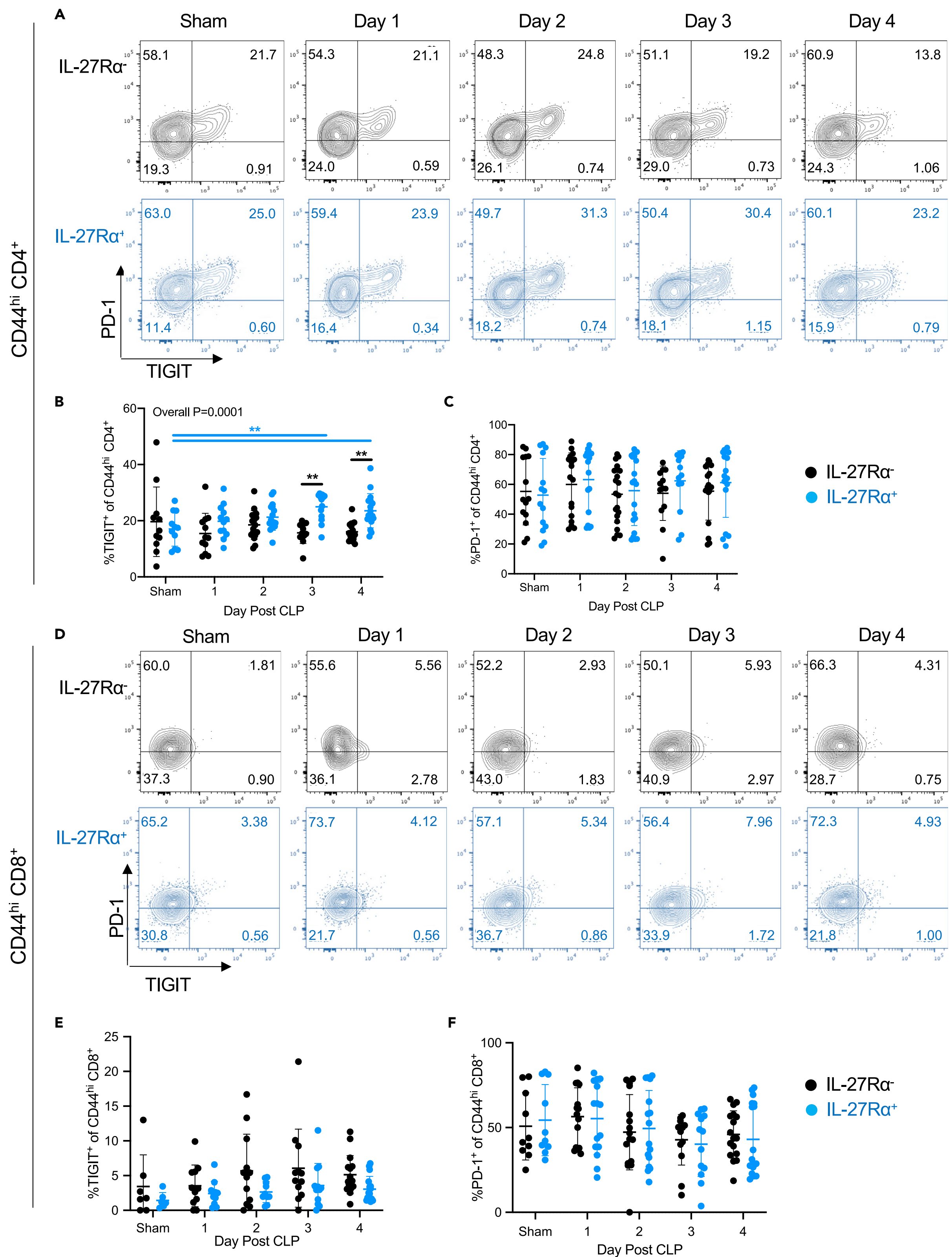

A
Sham
Day 1
Day 2
Day 3
Day 4
IL-27Rα⁻
IL-27Rα⁺
CD44hi CD4+
PD-1
TIGIT
B
Overall P=0.0001
%TIGIT+ of CD44hi CD4+
Day Post CLP
C
%PD-1+ of CD44hi CD4+
Day Post CLP
IL-27Rα⁻
IL-27Rα⁺
D
CD44hi CD8+
E
%TIGIT+ of CD44hi CD8+
Day Post CLP
F
%PD-1+ of CD44hi CD8+
Day Post CLP

Figure 2. IL-27Rα expression is associated with an increased frequency of TIGIT expression on memory CD4$^+$ T cells during sepsis

The gating strategy for PD-1 and TIGIT is shown in Figure S2.

(A) Representative flow cytometric plots showing PD-1 and TIGIT expression on CD44^{hi} memory CD4$^+$ T cells lacking (black) or expressing (blue) IL-27Rα in wild-type mice on days 1–4 after sham surgery ("sham") or CLP.

(B) The frequency of TIGIT$^+$ expressing IL-27Rα$^-$ (black) and IL-27Rα$^+$ (blue) CD44^{hi} memory CD4$^+$ T cells in sham and CLP wild-type mice on days 1–4 after surgery.

(C) The frequency of PD-1$^+$ expressing IL-27Rα$^-$ (black) and IL-27Rα$^+$ (blue) CD44^{hi} memory CD4$^+$ T cells in sham and CLP wild-type mice on days 1–4 after surgery.

(D) Representative flow cytometric plots showing TIGIT and PD-1 expression on CD44^{hi} memory CD8$^+$ T cells lacking (black) or expressing (blue) IL-27Rα in sham mice and CLP wild-type mice on days 1–4 after surgery.

(E) The frequency of TIGIT$^+$ expressing IL-27Rα$^-$ (black) and IL-27Rα$^+$ (blue) CD44^{hi} memory CD8$^+$ T cells in sham and CLP wild-type mice on days 1–4 after surgery.

(F) The frequency of PD-1$^+$ expressing IL-27Rα$^-$ (black) and IL-27Rα$^+$ (blue) CD44^{hi} memory CD8$^+$ T cells in sham and CLP wild-type mice on days 1–4 after surgery. Data were pooled from three independent experiments, with n = 7–17 mice per group. **$p < 0.01$. Error bars represent the mean ± the standard deviation.

sepsis (Figures 7A and 7B). The frequency of TNFα-producing CD4$^+$ cells was similar in sham and CLP mice (Figures 7A and 7C). Assessment of the CD44^{hi} CD4$^+$ T cells producing both IFN-γ and TNFα showed reduced frequencies of double-positive cells in the *Il27ra*$^{-/-}$ mice compared with the WT mice following sham surgery, but this difference did not persist after CLP-induced sepsis (Figure 7D). In the memory CD44^{hi} CD8$^+$ T cell compartment, WT and *Il27ra*$^{-/-}$ mice exhibited similar frequencies of IFN-γ$^+$ (Figures 7E and 7F), TNFα$^+$ (Figures 7E and 7G), and IFN-γ$^+$ TNFα$^+$ (Figures 7E and 7H) cells following CLP-induced sepsis. Circulating (plasma) IFN-γ was unchanged between wild-type versus Il27ra$^{-/-}$ mice that underwent sham surgery and CLP (Figure S3). Interestingly, circulating IFN-γ was reduced one and two days after CLP in wild-type mice (Figure S3).

To determine the impact of IL-27-induced TIGIT expression on the production of IFN-γ and TNFα, we assessed cytokine production in wild-type IL-27Rα$^+$ CD44^{hi} T cells. TIGIT expression was not associated with a difference in the frequency of IFNγ production by memory CD4$^+$ T cells in non-septic mice that underwent sham surgery or in septic mice (Figures 8A and 8B). However, the TIGIT$^-$ memory CD4$^+$ T cells in non-septic mice produced significantly more TNF than did TIGIT$^+$ cells (Figure 8C). The frequency of IFNγ$^+$ TNF$^+$ memory CD4$^+$ T cells was also elevated in the TIGIT$^-$ population of non-septic animals (Figure 8D). In the memory CD8$^+$ T cell compartment (Figure 8E), TIGIT expression was not associated with the frequency of IFNγ$^+$- (Figure 8F), TNF$^+$- (Figure 8G), or IFNγ$^+$ TNF$^+$ (Figure 8H)-producing cells. These data indicate that TIGIT expression is not associated with the defect in IFN-γ production by CD44^{hi} CD4$^+$ T cells in *Il27ra*$^{-/-}$ versus wild-type mice.

IL-27 signaling does not impact sepsis mortality

Our previous results indicated that IL-27 signaling is associated with an increase in TIGIT-expressing CD4$^+$ memory T cells during sepsis. To determine if these differences resulted in a difference in sepsis survival, we first compared the survival of mice deficient in *Il27ra* with wild-type mice. In this setting, there was no difference in sepsis mortality between *Il27ra*$^{-/-}$ and wild-type mice (Figure 9A). To confirm these results, we next used a monoclonal antibody specific to the p28 subunit of IL-27 (α-IL-27p28) to pharmacologically disrupt IL-27 signaling in wild-type mice. Mice that received the α-p28 monoclonal antibody exhibited similar survival compared with saline treated mice over the course of 7 days following CLP (Figure 9B). These results indicate that in a moderate mortality model of sepsis, IL-27 is not associated with worsened survival.

DISCUSSION

Based on previous reports investigating the role of IL-27 in cancer and chronic infections, we hypothesized that IL-27 signaling upregulates PD-1 and TIGIT on memory T cells during sepsis. Although IL-27Rα was associated with increased TIGIT expression on memory CD4$^+$ T cells following sepsis, this change did not correspond with a difference in T cell apoptosis, effector function, or sepsis survival.

Our results indicate that plasma IL-27 is significantly elevated following CLP, in agreement with previous work (Wirtz et al., 2006; Nelson et al., 2010; Wong et al., 2012, 2013, 2014; Bosmann et al., 2014b; Cao et al., 2014; Hanna et al., 2015; Gao et al., 2016; Yan et al., 2016). Because of previous studies linking IL-27 signaling to sepsis mortality, we sought to determine the impact of IL-27Rα signaling on memory T cells. Specifically, we sought to determine if there was an increase in IL-27Rα expression or changes in T cell apoptosis or cytokine production due to IL-27Rα. We found that the number of IL-27Rα$^+$ CD4$^+$

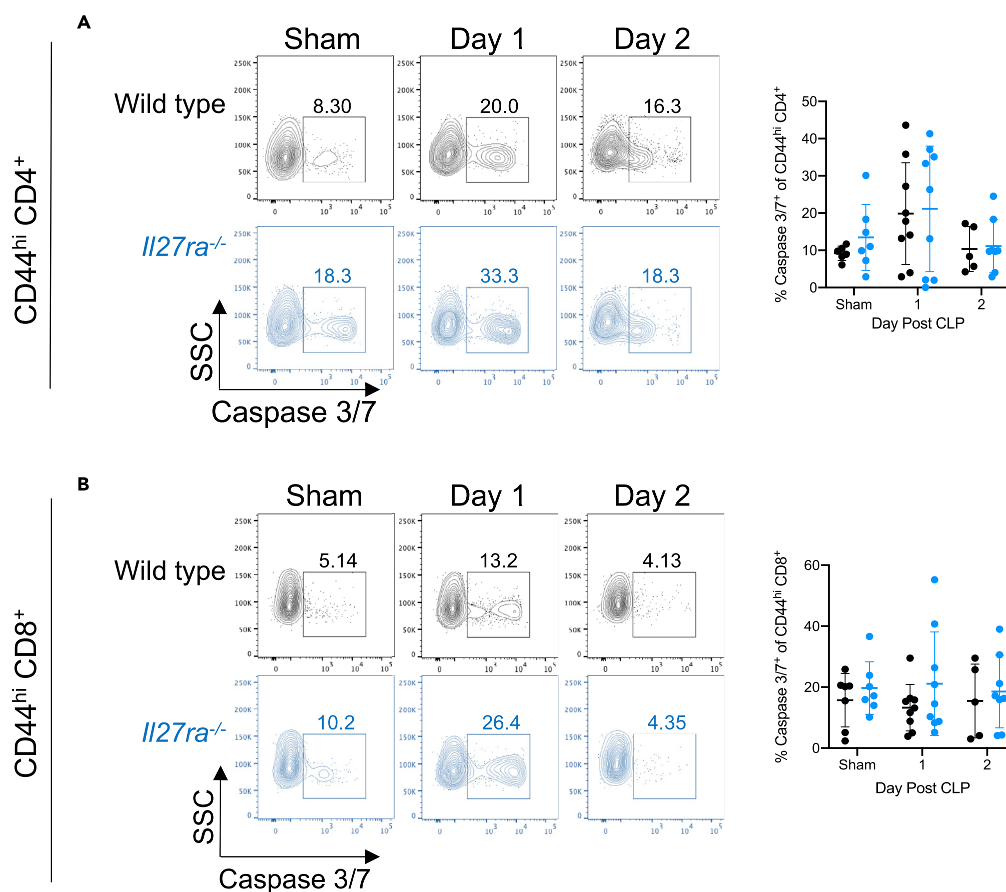


Figure 3. IL-27 signaling is not associated with CD44^{hi} T cell apoptosis in septic mice

(A) Representative flow cytometric plots showing Caspase 3/7 (x axis) by SSC (y axis) on CD44^{hi} memory CD4$^+$ T cells in wild-type and *Il27ra*$^{-/-}$ mice on days 1 and 2 after sham surgery ("sham") or CLP surgery (left). The frequency of apoptotic (Caspase 3/7$^+$) CD44^{hi} memory CD4$^+$ T cells is summarized on the right.

(B) Representative flow cytometric plots showing Caspase 3/7 (x axis) by SSC (y axis) on CD44^{hi} CD8$^+$ T cells after sham or CLP surgery in wild-type and *Il27ra*$^{-/-}$ mice on days 1 and 2 after surgery (left). The frequency of apoptotic (Caspase 3/7$^+$) CD44^{hi} memory CD8$^+$ T cells is summarized on the right. Data are representative of two experiments with n = 5–9 mice per group. Error bars represent the mean ± the standard deviation.

and CD8$^+$ memory T cells decreased following CLP, but this was not associated with an increase in IL-27Rα expression, T cell apoptosis, or alterations in cytokine production.

The link we observed between IL-27Rα and the upregulation of TIGIT on CD4$^+$ T cells during sepsis is consistent with previous papers that established a similar link in the setting of cancer and chronic infections (Chihara et al., 2018; Delong et al., 2019). In contrast to these studies, however, we did not find that IL-27 regulated TIGIT expression on CD8$^+$ T cells. This suggests that mechanisms other than IL-27 are responsible for the induction of TIGIT on CD8$^+$ T cells. Moreover, prior studies also reported a relationship between IL-27 and PD-1 expression on CD4$^+$ and CD8$^+$ T cells, which we also did not observe (Chihara et al., 2018; Delong et al., 2019). These studies highlight that the impact of IL-27 signaling on TIGIT expression may be disease state and context dependent.

Although our global knockout approach did not parse apart the cell-autonomous versus indirect effect of IL-27Rα signaling on TIGIT expression on CD4$^+$ memory T cells, previous studies have found that TIGIT expression is induced directly by IL-27 on T cells through the action of the transcription factors PRDM1 and c-Maf (Chihara et al., 2018). Another possibility is that IL-27 signaling on other cells subsequently results in the upregulation of TIGIT on CD4$^+$ T cells. As dendritic cells express IL-27Rα and also interact with T cells, it is possible that IL-27 signaling on DC may indirectly regulate TIGIT expression on T cells.

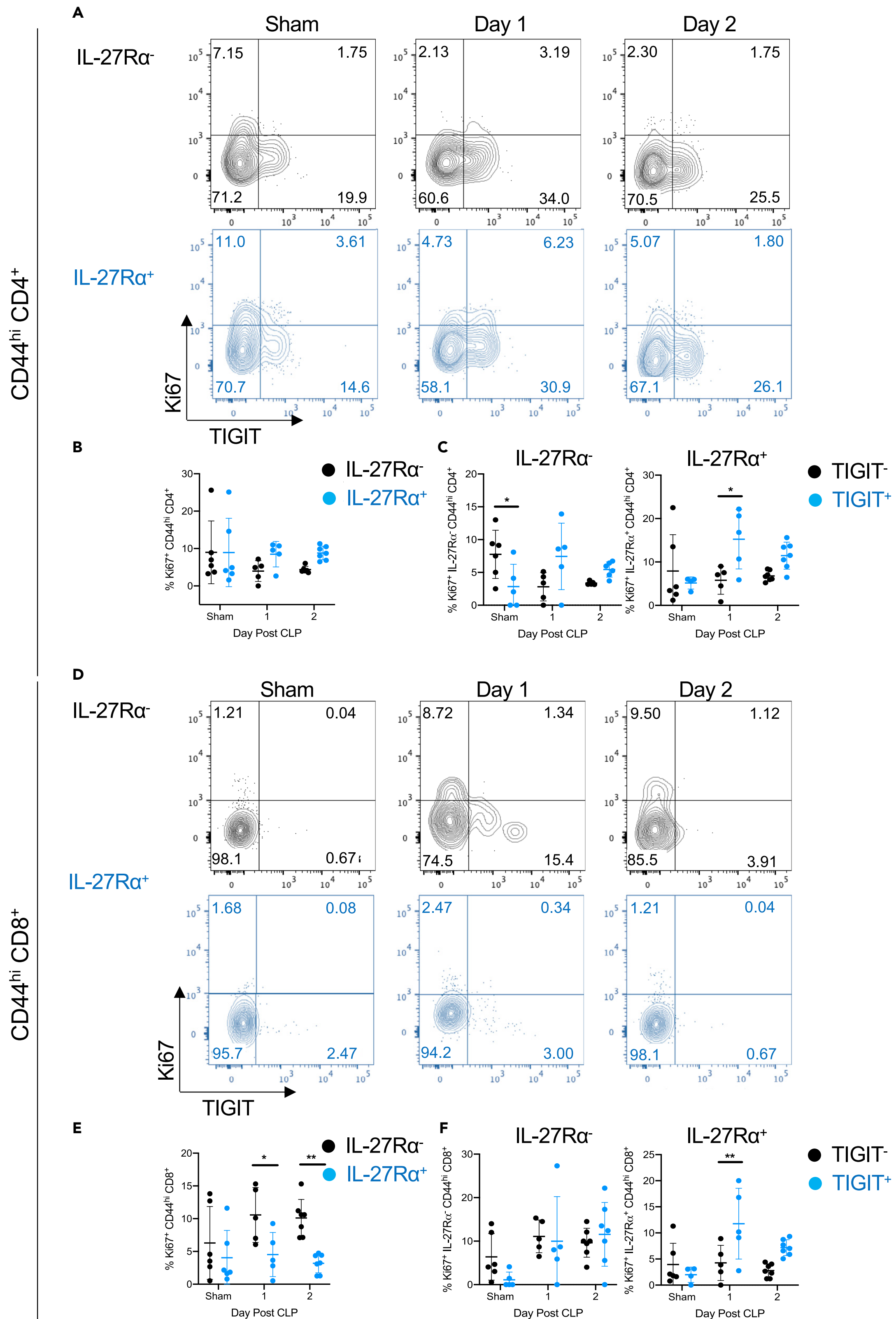

A
Sham
Day 1
Day 2
IL-27Rα-
IL-27Rα+
CD44hi CD4+
Ki67
TIGIT
7.15
1.75
71.2
19.9
2.13
3.19
60.6
34.0
2.30
1.75
70.5
25.5
11.0
3.61
70.7
14.6
4.73
6.23
58.1
30.9
5.07
1.80
67.1
26.1
B
% Ki67+ CD44hi CD4+
Day Post CLP
Sham
1
2
IL-27Rα-
IL-27Rα+
C
% Ki67+ IL-27Rα- CD44hi CD4+
% Ki67+ IL-27Rα+ CD44hi CD4+
TIGIT-
TIGIT+
D
CD44hi CD8+
1.21
0.04
98.1
0.67
8.72
1.34
74.5
15.4
9.50
1.12
85.5
3.91
1.68
0.08
95.7
2.47
2.47
0.34
94.2
3.00
1.21
0.04
98.1
0.67
E
% Ki67+ CD44hi CD8+
F
% Ki67+ IL-27Rα- CD44hi CD8+
% Ki67+ IL-27Rα+ CD44hi CD8+

Figure 4. IL-27 signaling is associated with reduced proliferation of CD44^{hi} CD8^{+} T cells in septic mice

Wild-type mice underwent sham ("sham") or CLP surgery, and splenocytes were harvested 1 to 2 days later for flow cytometric analysis.

(A) Representative flow cytometric plots showing TIGIT (x axis) versus Ki67 (y axis) expression in IL-27Rα^{-} (black) and IL-27Rα^{+} (blue) CD44^{hi} CD4^{+} T cells.

(B) Summary graph showing the frequency of Ki67^{+} CD44^{hi} CD4^{+} T cells between the IL-27Rα^{-} (black) and IL-27Rα^{+} (blue) populations.

(C) Summary graphs showing the frequency of Ki67^{+} CD44^{hi} CD4^{+} T cells within TIGIT^{-} (black) and TIGIT^{+} (blue) cells of the IL-27Rα^{-} (left) and IL-27Rα^{+} (right) populations.

(D) Representative flow cytometric plots showing TIGIT (x axis) versus Ki67 (y axis) expression in IL-27Rα^{-} (black) and IL-27Rα^{+} (blue) CD44^{hi} CD8^{+} T cells.

(E) Summary graph showing the frequency of Ki67^{+} CD44^{hi} CD8^{+} T cells between the IL-27Rα^{-} (black) and IL-27Rα^{+} (blue) populations.

(F) Summary graphs showing the frequency of Ki67^{+} CD44^{hi} CD8^{+} T cells within TIGIT^{-} (black) and TIGIT^{+} (blue) cells of the IL-27Rα^{-} (left) and IL-27Rα^{+} (right) populations. Data were pooled from two independent experiments with n = 5–7 per group. *p < 0.05, **p < 0.01. Error bars represent the mean $\pm$ the standard deviation.

One possible explanation for the association between IL-27 signaling and TIGIT expression and the *lack* of association between IL-27 signaling and PD-1 expression is the difference in TCR requirements for their expression. DeLong et al. noted that TCR signaling is required *in vitro* for IL-27 to upregulate the expression of TIGIT, but that TCR signaling was not required for IL-27 to upregulate PD-L1 (Delong et al., 2019). This suggests that TCR signaling in the earliest stages of sepsis could play a role in the ability of IL-27 to upregulate TIGIT. In contrast, the factors regulating IL-27-dependent expression of PD-1 are not involved in the early stages of sepsis. First, multiple pathways regulate the transcription of coinhibitory molecules within T cells. Although no literature exists specifically for TIGIT, Boss and colleagues have shown that *Pdcd1* expression is differentially regulated by the duration of antigen exposure (acute versus chronic) (Austin et al., 2014; Bally et al., 2016). In this sepsis model, we were only able to assess the T cell phenotype in settings of acute antigen exposure. It is possible that after longer periods, we would observe the changes noted in previous studies assessing the role of IL-27 in cancer and chronic infection. Despite little known information on the impact of antigen exposure and TIGIT, we observed an association between IL-27 signaling and TIGIT expression at early time points during sepsis. This indicates that acute antigen exposure allows a small but significant number of memory CD4^{+} T cells to upregulate the co-inhibitory molecule TIGIT. Surprisingly, these effects did not extend to memory CD8^{+} T cells, something that was previously seen in murine models of chronic infection and cancer. Because sepsis is primarily associated with the activation of CD4^{+} compared with CD8^{+} T cells, the difference in activation may explain why TIGIT was not upregulated on memory CD8^{+} T cells (Patenaude et al., 2005; Mcdunn et al., 2006).

Previous studies linked memory T cell apoptosis to sepsis mortality and the reactivation of latent viral infections in septic humans and mice. Because of the relationship between IL-27 signaling and TIGIT expression, we hypothesized that IL-27 is responsible for memory T cell apoptosis observed during sepsis. Surprisingly, we did not observe any difference in the frequency of apoptotic (Caspase 3/7^{+}) memory T cells between wild-type and *Il27ra*$^{-/-}$ mice despite the differences in TIGIT expression. These results are not necessarily surprising, as TIGIT is upregulated in a TCR-signaling-dependent mechanism (Delong et al., 2019), and TCR signaling does not lead to the T cell apoptosis observed during sepsis (Unsinger et al., 2006).

In this study, we looked specifically at memory T cells due to the link between sepsis-impaired memory T cells and increased long-term mortality (Duong et al., 2014; Chen et al., 2017; Xie et al., 2019b). In previous studies, IL-27 limited the response of activated and memory T cells (Pflanz et al., 2002; Villarino et al., 2003; Yoshimura et al., 2006; Gwyer Findlay et al., 2014). In line with these results, we found that the frequency of TIGIT^{+} memory CD4^{+} T cells in *Il27ra*$^{-/-}$ mice was significantly reduced 1 and 2 days after CLP. However, the frequency of TIGIT-expressing memory CD4^{+} T cells in both wild-type and *Il27ra*$^{-/-}$ mice was similar in mice that underwent sham surgery, suggesting that IL-27 is not required for the induction of TIGIT on memory T cells. In addition, the changes in TIGIT expression induced by sepsis were not associated with alterations in T cell effector function, as the reduction in IFNγ seen in *Il27ra*$^{-/-}$ memory CD4^{+} T cells seen after CLP was also present in mice that underwent sham surgery. These findings are consistent with previous studies that found IL-27 signaling induced expression of IFNγ through the transcription factor *T-bet* (Mayer et al., 2008; Villarino et al., 2003). Interestingly, a more recent study found that IFNγ expression was no different in the *Il27ra*$^{-/-}$ mice with chronic LCMV infection (Harker et al., 2018), suggesting a disease-state- and context-dependent role of IL-27 signaling in mediating IFNy production.

Multiple previous studies reported a significant increase in sepsis mortality caused by IL-27 signaling. Similar to the present study, these studies used pharmacological inhibitors of IL-27 signaling to measure the impact IL-27 has on sepsis survival (Wirtz et al., 2006; Bosmann et al., 2014a, 2014b). The other studies reported impressive decreases in sepsis mortality following pharmacological inhibition of IL-27 signaling, suggesting a major role for

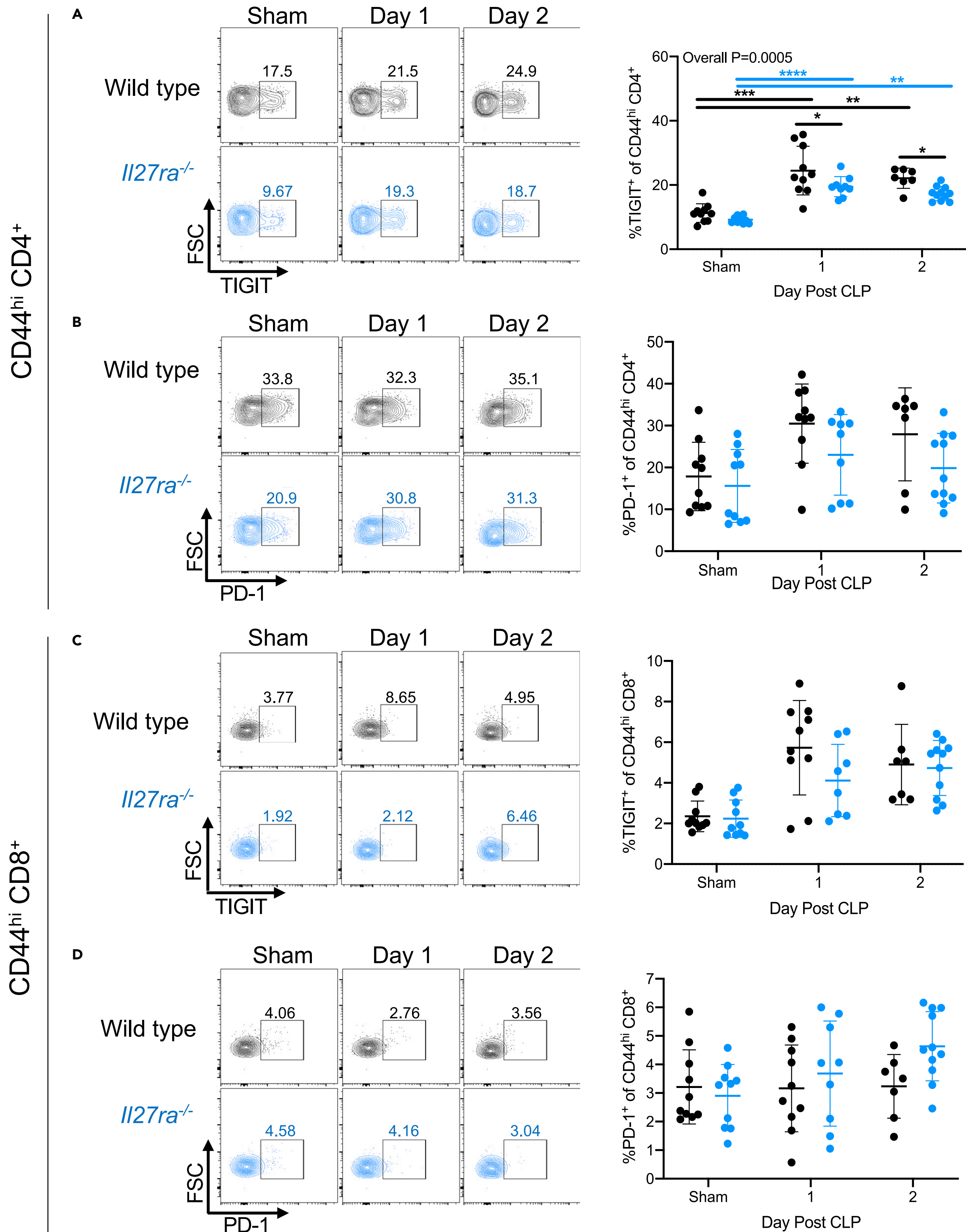

CD44hi CD4+
A
Sham
Day 1
Day 2
Wild type
17.5
21.5
24.9
Il27ra-/-
9.67
19.3
18.7
FSC
TIGIT
%TIGIT+ of CD44hi CD4+
Overall P=0.0005
****
**
***
**
*
*
Sham
1
2
Day Post CLP
B
Sham
Day 1
Day 2
Wild type
33.8
32.3
35.1
Il27ra-/-
20.9
30.8
31.3
FSC
PD-1
%PD-1+ of CD44hi CD4+
Sham
1
2
Day Post CLP
CD44hi CD8+
C
Sham
Day 1
Day 2
Wild type
3.77
8.65
4.95
Il27ra-/-
1.92
2.12
6.46
FSC
TIGIT
%TIGIT+ of CD44hi CD8+
Sham
1
2
Day Post CLP
D
Sham
Day 1
Day 2
Wild type
4.06
2.76
3.56
Il27ra-/-
4.58
4.16
3.04
FSC
PD-1
%PD-1+ of CD44hi CD8+
Sham
1
2
Day Post CLP

Figure 5. *Il27ra$^{-/-}$* septic mice have a reduced frequency of TIGIT^{+} memory CD4^{+} T cells

Wild-type and *Il27ra$^{-/-}$* mice underwent sham surgery ("sham") or CLP surgery. On days 1 and 2 following surgery, splenocytes were collected and used for flow cytometric analysis.

(A) Representative flow cytometric plots (left) and summary graphs (right) showing TIGIT expression (x axis) in CD44^{hi} memory CD4^{+} T cells of wild-type (top) and *Il27ra$^{-/-}$* (bottom) mice.

(B) Representative flow cytometric plots (left) and summary graphs (right) showing PD-1 expression (x axis) in CD44^{hi} memory CD4^{+} T cells of wild-type (top) and *Il27ra$^{-/-}$* (bottom) mice.

(C) Representative flow cytometric plots (left) showing TIGIT expression (x axis) in CD44^{hi} memory CD8^{+} T cells of wild-type (top) and *Il27ra$^{-/-}$* (bottom) mice.

(D) Representative flow cytometric plots (left) and summary graphs (right) showing PD-1 expression (x axis) in CD44^{hi} memory CD8^{+} T cells of wild-type (top) and *Il27ra$^{-/-}$* (bottom) mice. Data were pooled from two independent experiments, with n = 7–11 mice per group. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Error bars represent the mean ± the standard deviation.

IL-27 in sepsis mortality. We did not observe a similar decrease in mortality following IL-27 blockade. This may be linked to significant differences in the severity of the sepsis model used, as well as the reagents used. Although the previous studies used a high mortality CLP (where the vast majority of mice do not survive), our model used a mild-to-moderate mortality in which 50% or more mice are expected to survive. Thus, our data do not negate the possible role of IL-27 in high-mortality models of sepsis. Another difference in our study compared with the previous studies are the reagents used to interrupt IL-27 signaling: previous studies used a polyclonal α-IL-27 antibody (Bosmann et al., 2014a) or a soluble IL-27Rα (Wirtz et al., 2006). In contrast, we used a monoclonal α-IL-27 neutralizing antibody. Furthermore, our study compared the survival of *Il27ra$^{-/-}$* mice with that of wild-type mice using the cecal ligation and puncture model of sepsis. In contrast, a previous study comparing wild-type with Il27ra$^{-/-}$ mice during sepsis used a model of severe endotoxemia (Bosmann

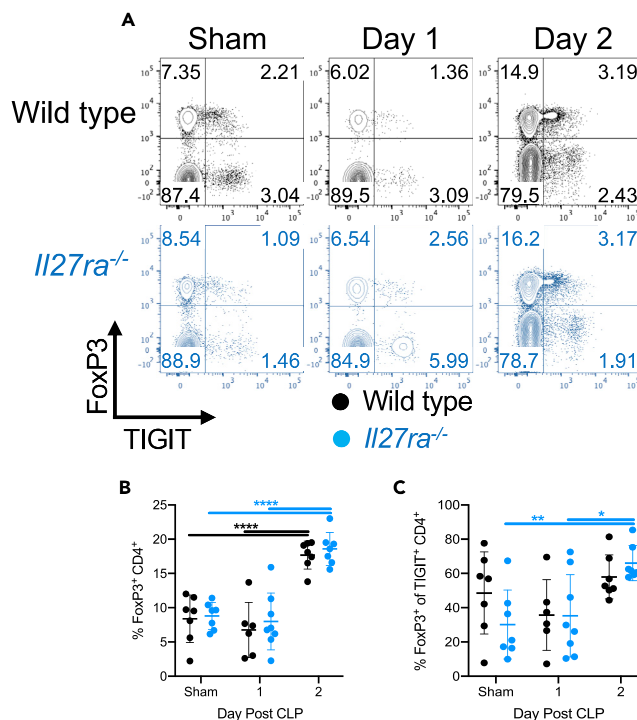


Figure 6. IL-27 signaling does not impact the frequency of Tregs during sepsis

One and two days after sham or CLP surgery on wild-type and *Il27ra$^{-/-}$* mice, splenocytes were collected and used for flow cytometric analysis.

(A) Representative flow plots showing TIGIT (x axis) versus FoxP3 (y axis) in wild-type (black) and *Il27ra$^{-/-}$* (blue) mice that underwent sham surgery (sham) or CLP 1 or 2 days prior.

(B) Summary graph showing the frequency of FoxP3^{+} CD4^{+} T cells within wild-type (black) or *Il27ra$^{-/-}$* (blue) mice following surgery.

(C) Summary graph showing the frequency of FoxP3^{+} cells within the TIGIT^{+} CD4^{+} compartments of wild-type (black) and *Il27ra$^{-/-}$* (blue) mice following sham or CLP surgery. Data were pooled from two independent experiments with n = 6–8 per group. *p < 0.05, **p < 0.01, ****p < 0.0001. Error bars represent the mean ± the standard deviation.

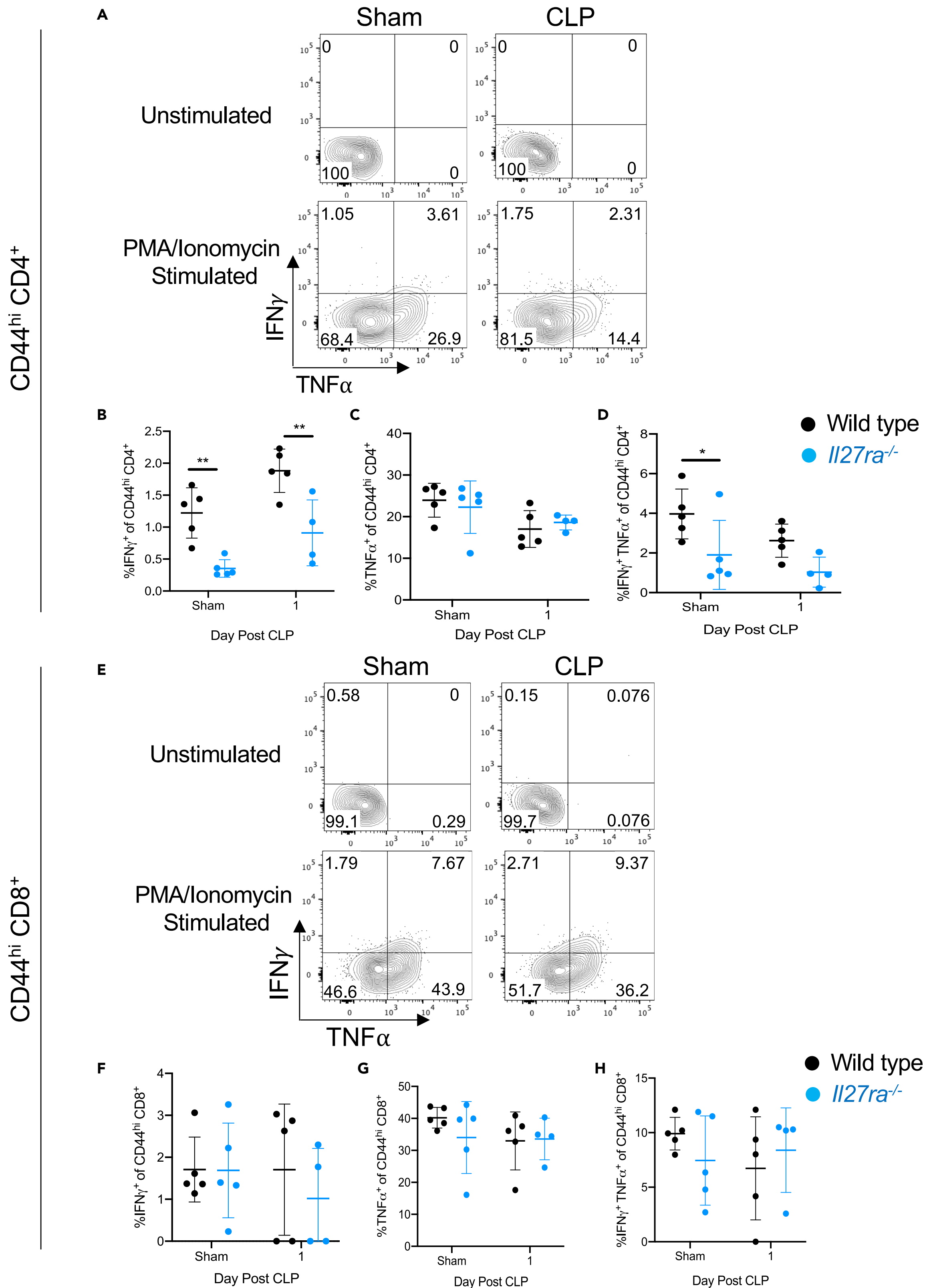

A
Sham
CLP
Unstimulated
PMA/Ionomycin Stimulated
IFNγ
TNFα
CD44hi CD4+
B
C
D
Wild type
Il27ra-/-
Day Post CLP
Sham
1
E
CD44hi CD8+
F
G
H

Figure 7. Memory CD4$^+$ T cells in *Il27ra$^{-/-}$* mice exhibit impaired production of IFN-γ at baseline and during sepsis

CLP and sham surgery ("sham") were performed on wild-type and *Il27ra$^{-/-}$* mice. One day after surgery, splenocytes were harvested for stimulation with PMA/Ionomycin or incubated without stimulation. Following incubation, samples were stained for IFN-γ and TNF-α and assessed by flow cytometry.

(A) Representative flow cytometric plots of IFN-γ (y axis) and TNF-α (x axis) production by the CD44^{hi} memory CD4$^+$ T cells of wild-type sham (left) and wild-type CLP mice (right) one day following surgery. The top row shows unstimulated controls, and bottom row shows stimulated samples.

(B) The frequency of IFN-γ producing CD44^{hi} memory CD4$^+$ T cells of wild-type (black) and *Il27ra$^{-/-}$* (blue) mice following surgery.

(C) The frequency of TNF-α-producing CD44^{hi} memory CD4$^+$ T cells of wild-type (black) and *Il27ra$^{-/-}$* (blue) mice following surgery.

(D) The frequency of IFN-γ and TNF-α co-producing CD44^{hi} memory CD4$^+$ T cells of wild-type (black) and *Il27ra$^{-/-}$* (blue) mice following surgery.

(E) Representative flow cytometric plots of IFN-γ (y axis) and TNF-α (x axis) production by the CD44^{hi} memory CD8$^+$ T cells of wild-type sham (left) and wild-type CLP mice (right) one day following surgery. The top row shows unstimulated controls, and bottom row shows stimulated samples.

(F) The frequency of IFN-γ-producing CD44^{hi} memory CD8$^+$ T cells of wild-type (black) and *Il27ra$^{-/-}$* (blue) mice following surgery.

(G) The frequency of TNF-α-producing CD44^{hi} memory CD8$^+$ T cells of wild-type (black) and *Il27ra$^{-/-}$* (blue) mice following surgery.

(H) The frequency of IFN-γ and TNF-α co-producing CD44^{hi} memory CD8$^+$ T cells of wild-type (black) and *Il27ra$^{-/-}$* (blue) mice following surgery. Data are representative of one experiment with n = 4–5 mice per group. *$p < 0.05$, **$p < 0.01$. Error bars represent the mean ± the standard deviation.

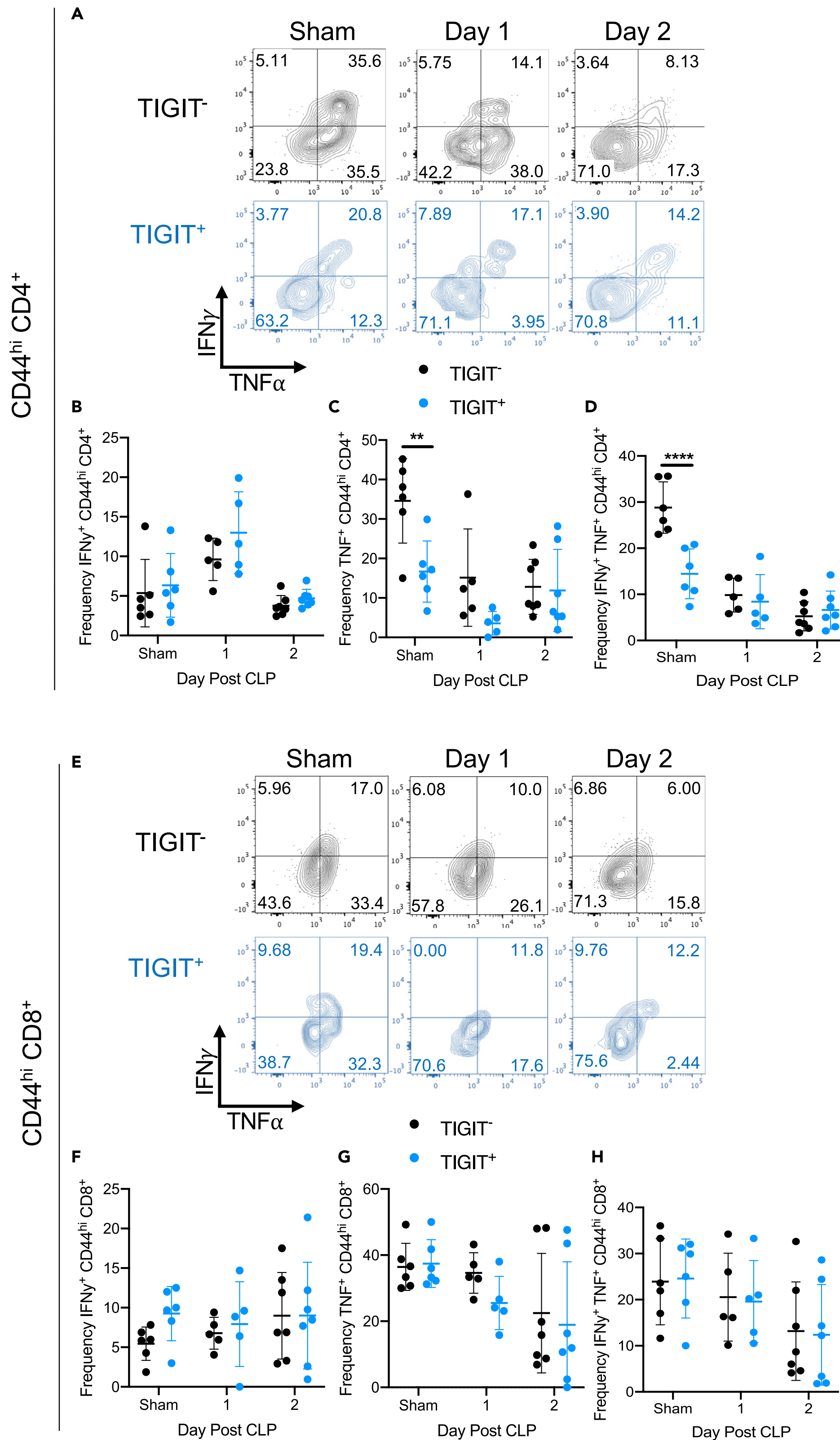
et al., 2014b), which may not adequately replicate the inflammation and immunological response of septic patients (Opal et al., 2014; Osuchowski et al., 2018).

Our results indicate that sepsis decreases the frequency and number of IL-27Rα expressing memory T cells. We hypothesized that the decrease in the IL-27Rα$^+$ memory T cell population could occur independently from apoptosis via a TIGIT-mediated inhibition of T cell proliferation (Joller et al., 2011). However, we did not observe a difference in the frequency of proliferating IL-27Rα$^-$ versus IL-27Rα$^+$ memory CD4$^+$ T cells, although TIGIT expression was associated with increased proliferation in IL-27Rα$^+$ cells. IL-27Rα$^+$ memory CD8$^+$ T cells had a lower frequency of proliferating cells compared with the IL-27Rα$^-$ population, but TIGIT expression was again associated with a higher frequency of proliferation. These data suggest that TIGIT does not inhibit memory T cell proliferation in septic mice. One possibility for the reduction in the IL-27Rα$^+$ memory T cell population is that these cells are leaving the circulation and going to the site of infection (Unsinger et al., 2010). In addition, IL-27Rα expression was associated with an increase in the expression of TIGIT on CD4$^+$ memory T cells in septic mice. Differences in antigen chronicity, TCR signaling, and the cytokine milieu between sepsis and these models may explain this. Future studies investigating the role of each of these factors will be important to establish what is responsible for the upregulation of co-inhibitory markers during sepsis.

Limitations of the study

Our results indicate that plasma IL-27 is significantly elevated following CLP, in agreement with previous work (Wirtz et al., 2006; Nelson et al., 2010; Wong et al., 2012, 2013, 2014; Bosmann et al., 2014b; Cao et al., 2014; Hanna et al., 2015; Gao et al., 2016; Yan et al., 2016). However, this increase did not correlate with an increase in the number of T cells expressing IL-27Rα. We found that both the frequency and number of CD44^{lo} naive and CD44^{hi} memory CD4$^+$ T cells expressing IL-27Rα were reduced following CLP compared with sham mice. We further found that the number of CD44^{hi} CD8$^+$ T cells expressing IL-27Rα$^+$ decreased following sepsis. These findings are in line with published results showing that IL-27Rα can be shed from the cell surface of activated T cells through the action of metalloproteases (Dietrich et al., 2014). Thus, increased metalloprotease-mediated shedding of cell surface IL-27Rα during sepsis might underlie this observation. The reduction in surface expression of IL-27Rα is in contrast to previous research on the role of IL-27 during toxoplasma infection (Villarino et al., 2005). Villarino et al. found that mice infected with toxoplasma have a significantly higher frequency of both CD4$^+$ and CD8$^+$ T cells expressing IL-27Rα compared with uninfected mice (Villarino et al., 2005). Because the immune response to parasitic infection is very distinct from what occurs during sepsis, our results suggest that sepsis differentially regulates IL-27Rα expression.

Previous studies of co-inhibitory receptor expression on T cells following CLP have revealed that co-inhibitory receptor expression remains elevated far longer than expected from T cell activation and is associated with impairments in T cell effector function and ultimately apoptosis. However, TIGIT may also be acting as an activation marker. We cannot conclude whether it was acting as an activator or suppressor of T cell function. In addition, the changes in the frequency of TIGIT expression on T cells in this study were quite small, and the biological significance of these changes is unclear. Few studies have assessed the impact of TIGIT on T cell responses in the context of sepsis, but previous studies in our laboratory have found a similar induction of TIGIT expression in the CD4$^+$ T cells of septic mice with solid tumors (Chen et al., 2019). However, future studies addressing the physiological impact of TIGIT on survival from sepsis are necessary to better understand the biological relevance of these findings.


A
CD44hi CD4+
Sham
Day 1
Day 2
TIGIT-
TIGIT+
5.11
35.6
23.8
35.5
5.75
14.1
42.2
38.0
3.64
8.13
71.0
17.3
3.77
20.8
63.2
12.3
7.89
17.1
71.1
3.95
3.90
14.2
70.8
11.1
IFNγ
TNFα
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D
Frequency IFNγ+ CD44hi CD4+
Frequency TNF+ CD44hi CD4+
Frequency IFNγ+ TNF+ CD44hi CD4+
Day Post CLP
Sham
1
2
**
****
E
CD44hi CD8+
5.96
17.0
43.6
33.4
6.08
10.0
57.8
26.1
6.86
6.00
71.3
15.8
9.68
19.4
38.7
32.3
0.00
11.8
70.6
17.6
9.76
12.2
75.6
2.44
F
G
H
Frequency IFNγ+ CD44hi CD8+
Frequency TNF+ CD44hi CD8+
Frequency IFNγ+ TNF+ CD44hi CD8+

Figure 8. TIGIT is not associated with the impairments observed in cytokine production

CLP and sham surgery ("sham") were performed on wild-type mice. One and two days after surgery, splenocytes were harvested for stimulation with PMA/Ionomycin or incubated without stimulation. Following incubation, samples were stained for TIGIT, IFN-γ, and TNF-α and assessed by flow cytometry.

(A) Representative flow cytometric plots of IFN-γ (y axis) and TNF-α (x axis) production by $TIGIT^-$ (black) and $TIGIT^+$ (blue) $CD44^{hi}$ memory $CD4^+$ T cells following surgery.

(B) The frequency of IFN-γ producing $TIGIT^-$ (black) and $TIGIT^+$ (blue) $CD44^{hi}$ memory $CD4^+$ T cells following surgery.

(C) The frequency of TNF-α producing $TIGIT^-$ (black) and $TIGIT^+$ (blue) $CD44^{hi}$ memory $CD4^+$ T cells following surgery.

(D) The frequency of IFN-γ and TNF-α co-producing $TIGIT^-$ (black) and $TIGIT^+$ (blue) $CD44^{hi}$ memory $CD4^+$ T cells following surgery.

(E) Representative flow cytometric plots of IFN-γ (y axis) and TNF-α (x axis) production by $TIGIT^-$ (black) and $TIGIT^+$ (blue) $CD44^{hi}$ memory $CD8^+$ T cells of wild-type mice following sham or CLP surgery.

(F) The frequency of IFN-γ-producing $TIGIT^-$ (black) and $TIGIT^+$ (blue) $CD44^{hi}$ memory $CD8^+$ T cells following surgery.

(G) The frequency of TNF-α-producing $TIGIT^-$ (black) and $TIGIT^+$ (blue) $CD44^{hi}$ memory $CD8^+$ T cells following surgery.

(H) The frequency of IFN-γ and TNF-α co-producing $TIGIT^-$ (black) and $TIGIT^+$ (blue) $CD44^{hi}$ memory $CD8^+$ T cells following surgery. Data are representative of two experiments with n = 5–7 mice per group. $^{**}p < 0.01$, $^{****}p < 0.0001$. Error bars represent the mean ± the standard deviation.

Although T cell exhaustion is observed early after the onset of sepsis in both mouse models and human patients, it is unclear if the factors that regulate T cell exhaustion in early stages are the same that maintain T cell exhaustion in the weeks to months following sepsis resolution. Future studies that look at these factors will be necessary. Another limitation of this study was the use of immunologically naive mice, which have a lower frequency of memory T cells than do humans. We have previously published data indicating that mice sequentially infected with viral and bacterial infections have a memory compartment that better reflects what is observed in humans (Xie et al.,

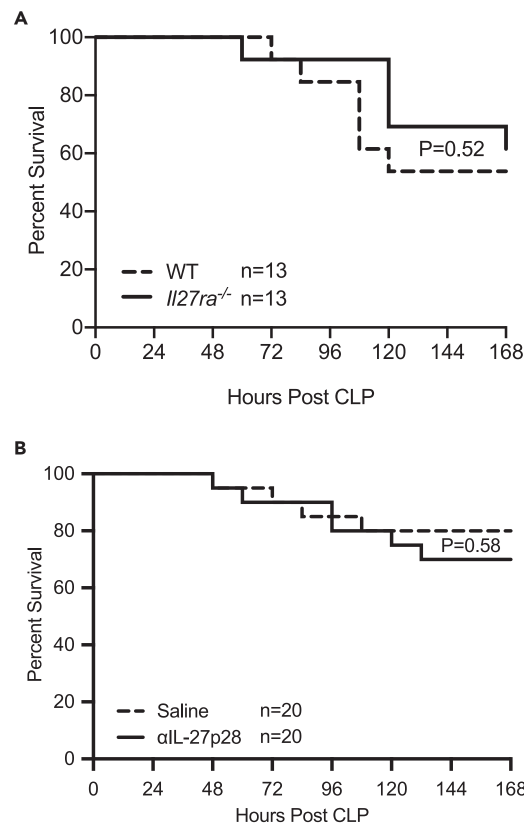

Figure 9. IL-27 signaling does not impact the survival of septic mice

(A) Wild-type (WT, dotted line) and *Il27ra*$^{-/-}$ (KO, solid line) mice underwent CLP and were followed for 7 days for survival. Each group contained 13 animals and was age and gender matched.

(B) Wild-type mice underwent CLP and received either saline (dotted line) or anti-IL-27 neutralizing mAb (solid line). Mice were followed for survival for 7 days following surgery. Each group contained 20 animals and was age and gender matched. All data shown are pooled from 2–3 independent experiments.

2019a). Although we failed to observe an association between IL-27 signaling and PD-1 expression in previously healthy septic mice, the findings in mice with pre-existing cancer may be different because there is an association between IL-27 signaling and PD-1 expression in the setting of cancer. In line with this idea, previous research in our laboratories has found that cancer septic mice differ in the expression of co-inhibitory markers on their T cells compared with previously healthy septic mice (Fox et al., 2010; Lyons et al., 2016; Xie et al., 2018; Chen et al., 2019).

Resource availability

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Mandy Ford (mandy.ford@emory.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This study did not generate/analyze datasets or code.

METHODS

All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2021.102093.

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AUTHORS CONTRIBUTION

Conceptualization: K.N.M., C.M.C., and M.L.F.; Formal Analysis: K.N.M., C.M.C., and M.L.F.; Investigation: K.N.M., Z.L., M.X., D.B.C., Y.S., and C.C.; Writing—Original Draft: K.N.M.; Writing—Review and Editing: K.N.M., C.M.C., and M.L.F.; Visualization: K.N.M., C.M.C., and M.L.F.; Supervision: C.M.C. and M.L.F.; Funding Acquisition: C.M.C. and M.L.F.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Supplemental Information

The IL-27 receptor regulates TIGIT on memory CD4^{+} T cells during sepsis

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Supplemental Figures

Figure S1

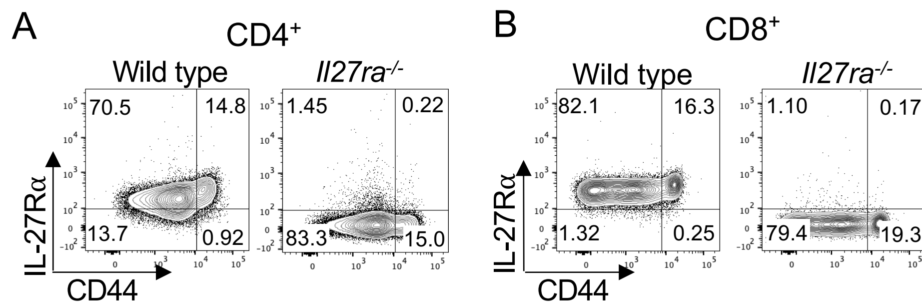

Figure S1: Gating strategy for IL-27Rα on CD4⁺ and CD8⁺ T cells (Related to Figure 1)
Splenocytes were obtained from wild type or *Il27ra$^{-/-}$* mice and stained for IL-27Rα and CD44. (**A**) Representative flow cytometric plots showing IL-27Rα (y-axis) vs CD44 (x-axis) for $CD4^+$ T cells in wild type (left) and *Il27ra$^{-/-}$* (right) mice. (**B**) Representative flow cytometric plots showing IL-27Rα (y-axis) vs CD44 (x-axis) for $CD4^+$ T cells in wild type (left) and *Il27ra$^{-/-}$* (right) mice.

Figure S2

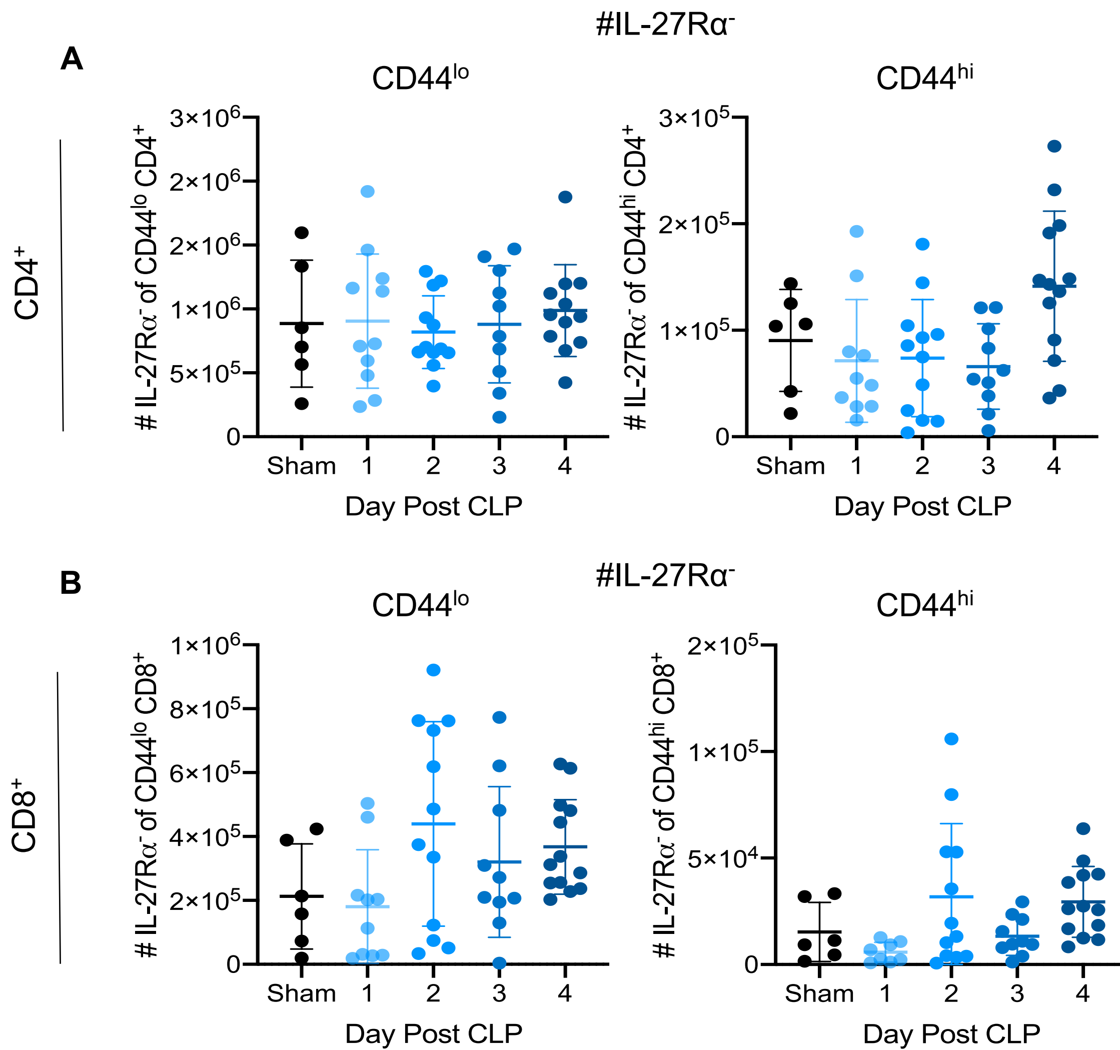

Figure S2: IL-27Ra- CD44lo and CD44hi numbers are unchanged following sepsis (Related to Figure 1). Following cecal ligation and puncture (CLP) or sham surgery (sham), animals were euthanized on the indicated days. Spleens were harvested for analysis by flow cytometry on days 1 through 4. **(A)** The absolute number of $CD4^+$ $CD44^{lo}$ naïve (left) and $CD4^+$ $CD44^{hi}$ memory (right) T cells *not* expressing IL-27Rα in sham and CLP mice on days 1-4 after surgery. **(B)** The absolute number of $CD8^+$ $CD44^{lo}$ naïve (left) and $CD8^+$ $CD44^{hi}$ memory (right) T cells *not* expressing IL-27Rα in sham and CLP mice on days 1-4 after surgery. All summary data was pooled from 3 independent experiments, with n=7-18 mice per group.

Figure S3

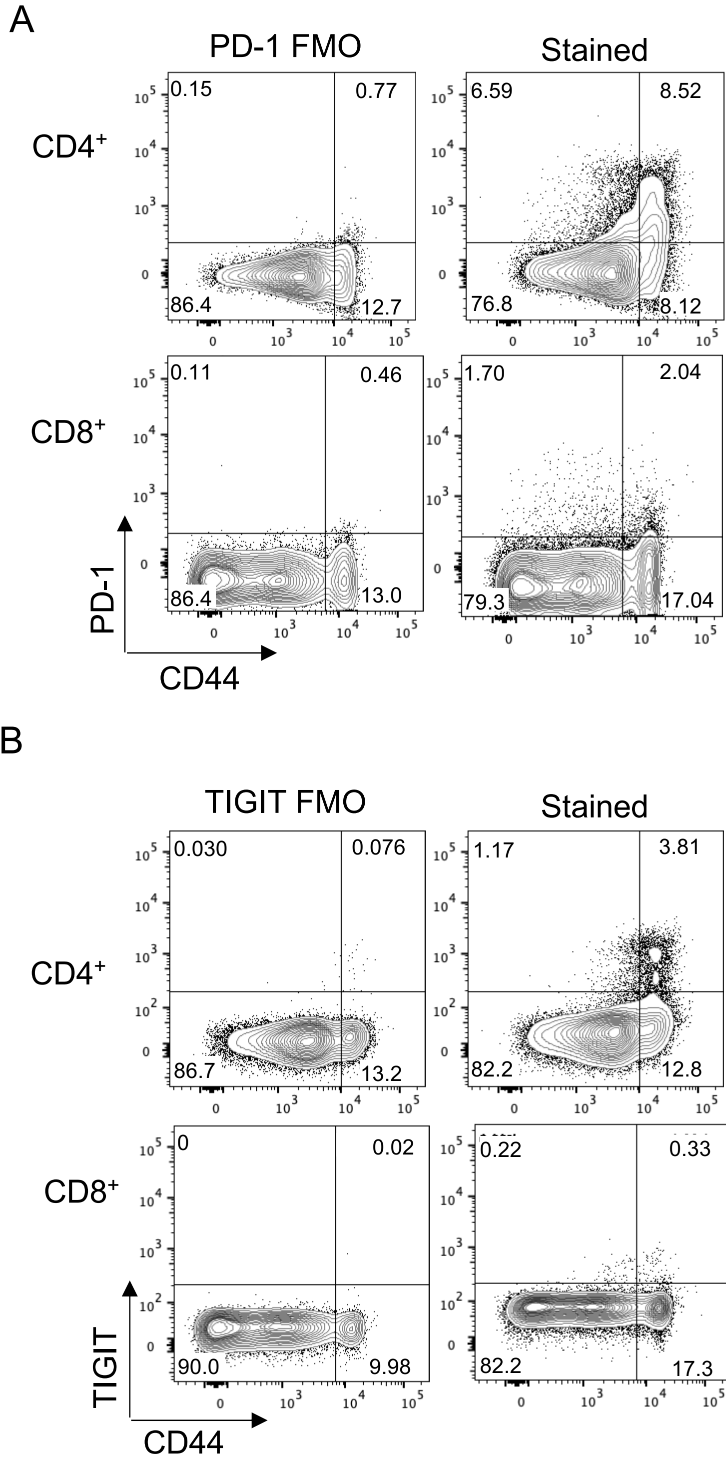

Figure S3: Gating strategy for PD-1 and TIGIT on CD4+ and CD8+ T cells (Related to Figure 2)

Splenocytes were obtained from wild type septic mice on day 4 following CLP and used for flow cytometric analysis. (**A**) Representative flow cytometric plots showing PD-1 (y-axis) vs CD44 (x-axis) using a PD-1 FMO (left) and stained (right) controls. CD4+ T cells are shown in the top series and CD8+ cells at bottom. **(B**) Representative flow cytometric plots showing TIGIT (y-axis) vs CD44 (x-axis) using a PD-1 FMO (left) and stained (right) controls. CD4+ T cells are shown in the top series and CD8+ cells at bottom.

Figure S4

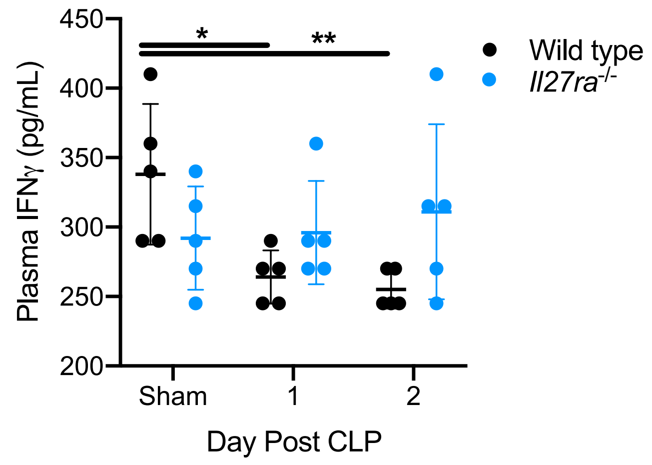

Figure S4: Circulating IFN*γ* is unchanged in *Il27ra$^{-/-}$* vs wild type mice following CLP (Related to Figure 7) Plasma was purified from the blood of wild type and *Il27ra$^{-/-}$* mice that underwent sham ("sham") or CLP surgery on days 1 and 2 after surgery.

Transparent Methods

Animals

Six to 12-week-old male and female gender matched mice (mean weight 20g) were used for all experiments. The wild type mice used for T cell phenotyping experiments were either C57BL/6J or C57BL/6NJ mice obtained from Jackson Laboratories (Bar Harbor, ME). Transgenic *Il27ra*$^{-/-}$ mice on a mixed C57BL/6NJ and 6NTac background were a gift from Dr. Jacob Kohlmeier (Emory University, Atlanta, Georgia; animals were originally obtained from Jackson Laboratories, Bar Harbor, ME). C57BL/6NJ (Jackson Laboratories, Bar Harbor, ME) mice were used as controls in experiments with transgenic *Il27ra*$^{-/-}$ mice. Mice obtained from external sources were acclimated for at least 72 hours prior to being used in experiments. Mice were randomly allocated to receive either sham surgery or cecal ligation and puncture (CLP, details below) and were either sacrificed between 24 and 96 hours after surgery or followed for 7 days to determine survival. All experiments were performed in accordance with the National Institutes of Health Guidelines for the Use of Laboratory Animals and were approved by the Emory University Institutional Animal Care and Use Committee (Protocol 201700361.RM001-EI-N). Mice were housed in specific pathogen free conditions with a 12-hour light cycle.

Cecal Ligation and Puncture (CLP)

Cecal ligation and puncture was performed as previously described (Chen et al., 2017, Chen et al., 2019). Surgeries were performed between the hours of 9am and 2pm to minimize the confounding effects of circadian rhythm. Prior to surgery, 0.1 mg/kg of buprenorphine (McKesson Medical, San Francisco, CA) was administered to each mouse subcutaneously (s.c.) to minimize suffering. Ophthalmic eye gel was administered to prevent corneal ulceration during surgery and abdominal fur was shaved to minimize risk of wound contamination. The anesthetic depth necessary for surgery was induced with inhaled isoflurane (3% in 100% O_2) and reduced to 2% after the surgical plane was reached. After skin disinfection, a midline incision was made, and the cecum was exteriorized. For mice in the control group (receiving sham surgery), the cecum was then replaced in the abdominal cavity, and the abdominal wall closed with 4-0 silk thread. If mice underwent cecal ligation and puncture, approximately 75% of the cecal length was ligated with nylon thread, before being punctured with a 25-gauge needle through and through. A small amount of stool was then gently expelled before the cecum was returned to the abdominal cavity. The abdominal wall was closed with 4-0 silk suture and the skin closed with veterinary glue. Immediately after surgery, 1mL of sterile saline was administered s.c. for fluid resuscitation in addition to 50 mg/kg ceftriaxone (Acros Organics, Morris Plains, NJ) and 35 mg/kg metronidazole (Sigma-Aldrich, St. Louis, MO) for pathogen control**.** Animals were then placed in a new cage on a warming pad and monitored for recovery. Following recovery, the cages were returned to their housing room. Antibiotics (same as above) were administered every 12 hours for the first 48 hours following surgery. All mice were monitored twice a day for the duration of the experiments and weighed every other day. Any mouse that lost 25% body weight or appeared moribund was humanely euthanized by asphyxiation with CO_2 or exsanguination after exposure to a high concentration of isoflurane followed by cervical dislocation. Moribund animals were defined by a) major organ failure or medical conditions unresponsive to treatment, b) surgical complications unresponsive to immediate intervention or c) clinical or behavioral signs unresponsive to appropriate intervention persisting for 24 hours. In experiments using neutralizing IL-27p28 antibody (clone: MM27.7B1, BioXCell), 500 μg was administered into the intraperitoneal cavity before abdominal wall closure following CLP or sham surgery.

ELISAs

Plasma samples were obtained via terminal heart puncture or saphenous vein collection into tubes containing EDTA. After centrifugation, the plasma layer was collected and cryopreserved until analysis. Samples were diluted using 1x PBS and assessed for IL-27 concentration using an anti-IL-27p28 ELISA kit (Invitrogen, Carlsbad, CA) or IFNγ concentration using an anti-IFNγ ELISA kit (Invitrogen, Carlsbad, CA) following manufacturer instructions. Results were analyzed using Four Parameter Logistic Regression.

Flow cytometry

On days 1-4 following CLP, mice from each group were randomly chosen for sacrifice. Spleens were harvested and strained through a 70 μM nylon filter before washing with cold 1x PBS through centrifugation. Splenocytes were subsequently resuspended in PBS and 2 million cells were used for staining. Prior to staining with target antibodies, all samples were stained with TruStain FcX anti-mouse CD16/32

(BioLegend, San Diego, CA) following manufacturer instructions. Cells were then stained for surface markers and incubated on ice for 25 minutes. After staining, samples were washed with MACS Buffer and resuspended in CountBright Absolute Counting Beads (Thermo Fisher Scientific, Waltham, MA) according to manufacturer instructions. The antibodies used for T cell exhaustion phenotyping are as follows: TIGIT-BV421 (Clone 1G9, BD), Live/Dead Aqua (Invitrogen), NK1.1-BV650 (clone PK136, BioLegend), CD44 on PerCP Cy 5.5 (clone IM7, BioLegend) or BUV737 (clone IM7, BD), IL-27Rα-PE (clone 2918, BD), PD-1-APC/Cy7 (clone 29F.1A12, BioLegend), CD4-BUV395 (clone GK1.5, BD), CD3e-BUV496 (clone 145-2C11, BD), and CD8a on BUV737 or BUV805 (both clone 53-6.7, BD). For caspase 3/7 staining, surface staining was done as described above with the following markers: CD4-BUV395 (clone GK1.5, BD), CD3e-BUV496 (clone 145-2C11, BD), CD8a on BUV737 (clone 53-6.7, BD), NK1.1-BV650 (clone PK136, BioLegend), and IL-27Rα-PE (clone 2918, BD).Cells were then resuspended in 1x PBS and Caspase-3/7 stain according to manufacturer instructions (CellEvent Caspase-3/7 Green Detection Reagent, Invitrogen, Carlsbad, CA). Samples were incubated for one hour at 37□ C and caspase 3/7 staining was immediately detected. For intracellular cytokine staining (ICCS), splenocytes were stimulated for 4 hours with 20 ng/mL PMA (Sigma-Aldrich) and 0.75 μg/mL Ionomycin (Sigma-Aldrich) in the presence of GolgiPlug (BD). Samples were subsequently surface stained as described above with CD4-BUV395 (clone GK1.5, BD), CD3e-BUV496 (clone 145-2C11, BD), CD8a on BUV737 (clone 53-6.7, BD), IL-27Rα-PE (clone 2918, BD). Following surface staining, the cells were fixed and permeabilized according to manufacturer's instructions (BD Fixation/Permeabilization Solution Kit). Cells were then stained with TNFα-APC and IFNγ-A700. For Treg and Ki67 (proliferation) staining, extracellular staining was done as above using CD4-BUV395 (clone GK1.5, BD), CD3e-BUV496 (clone 145-2C11, BD), CD8a on BUV737 (clone 53-6.7, BD), PD-1-APC/Cy7 (clone 29F.1A12, BioLegend), TIGIT-BV421 (Clone 1G9, BD), Live/Dead Aqua (Invitrogen), NK1.1-BV650 (clone PK136, BioLegend), and CD44-BUV737 (clone IM7, BD). Following extracellular staining, cells were fixed and permeabilized using the Foxp3/transcription staining buffer kit (eBioscience) according to manufacturer instructions. Cells were then stained with FoxP3-APC (clone FJK-16s, eBioscience) and Ki67-AF700 (clone 16A8, BioLegend). All samples were run on a LSRFortessa (BD Biosciences, San Jose, CA). All flow cytometric data was analyzed using FlowJo version 10.2 (BD, Ashland, OR). The FlowAI plugin (Monaco et al., 2016) found on FlowJo Exchange (www.flowjo.com/exchange) was used in sample pre-processing to eliminate artifacts caused by variable flow rate before analysis.

Statistics
All statistical analysis was performed using Prism 8.3.1 (GraphPad, San Diego, CA). Kruskal-Wallis tests with Dunn's multiple comparisons test was used when comparing within a single group longitudinally. When comparing between two groups at multiple time points, two-way ANOVA was used with Sidak's multiple comparison test. Survival curves were assessed using a Log-rank (Mantel-Cox) Test. Results are reported as the mean of each group ± SD. P-values ≤ 0.05 were considered statistically significant.

Supplemental References

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