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Role of T cells in a Murine Model of *E. coli* Sepsis.

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Summary

To study the role of T cells in gram-negative sepsis, we developed a mouse model in which intravenous injection of *E. coli* results in severe systemic illness, with high mortality rates after day 5. A large proportion of both CD4+ and CD8+ T cells are activated within 1 day after infection, as evidenced by up-regulation of CD69 and down-regulation of CD62L. Even more surprisingly, T cell deficient mice exhibit markedly decreased disease severity compared to wild-type mice, indicating a pathogenic role of T cells. Mice lacking IFNγ also show diminished disease, and exhibit reduced T cell activation. Therefore, the pathogenic role of T cells may be mediated by IFNγ. Both T-cell and IFNγ-deficient mice have reduced serum IL-6 levels compared to wild-type mice, suggesting that T cells may stimulate innate immune responses, resulting in enhancement of disease. These data indicate an important role for T cells in a mouse model of *E. coli* sepsis, and reveal an unexpected early and pathogenic T cell response to this bacterial infection.

Introduction

Sepsis continues to be a major burden on health care resources, affecting approximately 700,000 people per year in the U.S. often requiring extended hospitalization. In addition, mortality remains high at 25-30%, and specific therapies are lacking. This is in part due to incomplete understanding of the pathogenesis of the disease process. The concept of exacerbated inflammatory responses to overwhelming bacterial infection [1] appears to be an over-simplification. It has been established that activation of the innate immune system occurs early in the course of sepsis, resulting in release of a multitude of pro-inflammatory mediators. Although several studies have revealed correlations between the production of mediators and disease severity and/or mortality, clinical trials with anti-inflammatory agents have had disappointing results so far [2, 3]. Mortality in humans with sepsis is often delayed and the late phase of sepsis appears to be characterized by immunosuppression rather than overactivation [4]. It has been postulated that altered adaptive immune responses may play a role in the outcome of sepsis [2].

In order to study adaptive immune responses, which are traditionally thought to occur at least 3-4 days after antigen exposure, we developed an *E. coli* mouse model of gram-negative sepsis in which mortality is delayed past the first few days after introduction of the bacteria. We found that mice develop severe systemic illness upon infection with *E. coli*, with high mortality rates 5-7 days after infection. In this model, T cell activation occurred surprisingly early (within 24 hours), and absence of T cells was associated with diminished disease, as was

absence of IFNγ. We conclude that adaptive immune responses play a pathogenic role in this model of *E. coli* sepsis, possibly mediated by IFNγ.

Results

Intravenous administration of E. coli results in a dose dependent systemic disease in mice.

Initial experiments were designed to establish a model of systemic *E. coli* sepsis in mice. BALB/c mice were injected with either PBS or increasing doses of *E. coli,* and disease manifestations including weight changes and the appearance of ruffled fur, hunched appearance and lethargy were monitored daily (Figure 1). Mice inoculated with 1.5×10^7 CFU of *E. coli* exhibited mild weight loss on day 1 after infection ($P<0.01$ compared to PBS injected mice), with recovery to baseline weights over subsequent days. Mice infected with 1.5×10^8 CFU demonstrated progressive weight loss with high mortality rates (30%) after day 5. Weight loss was significantly worse in mice inoculated with 1.5×10^8 CFU of *E. coli* compared to those given an inoculation dose of 1.5×10^7 CFU ($P<0.01$ on day 1 after infection, $P<0.001$ on subsequent days). In concordance with weight loss, mice inoculated with 1.5×10^7 CFU of *E. coli* developed mild and transient outward signs of disease as shown by disease scores ($P<0.01$ on day 1 after infection compared to PBS group, not significant on subsequent days). Mice inoculated with 1.5×10^8 CFU had a marked hunched and ruffled appearance as early as day 1 after infection ($P<0.01$ compared to mice inoculated with 1.5×10^7 CFU), which became progressively worse with notable lethargy in most animals by day 4 after infection. An inoculation dose greater than 3×10^8 CFU resulted in early death (days 1-2) in the majority of animals (data not shown). In mice infected with 1.5×10^8 CFU of *E. coli*, cultures of kidney, liver, lung and spleen

homogenates were positive throughout the course of infection, whereas blood cultures were positive during the first 2-3 days only (data not shown). Thus, intravenous injection of *E. coli* resulted in a dose-dependent systemic disease with evidence of spread of the bacteria to multiple organs. A similar dose-response and disease course were seen in C57BL/6 mice (data not shown).

Activation of T cells in E. coli infected mice.

To examine whether T cells play a role in this mouse model of sepsis, we first assessed whether there was evidence of T cell activation upon systemic *E. coli* infection. Splenocytes and peripheral lymph nodes from *E. coli*-infected mice were isolated at various time points after infection and analyzed by staining and flow cytometry for the expression of activation markers. In comparison to PBS-injected mice, T cells from *E. coli*-infected mice exhibited increased expression of CD69 and decreased expression of CD62L, indicating T cell activation (Figure 2). This pattern of expression was noted on both CD4+ and CD8+ cell populations. Increased expression of CD69 was present remarkably early (within 24 hours) of *E. coli* infection on a surprisingly large proportion of T cells (up to 68% of CD4+ cells and up to 82% of CD8+ cells, $P<0.05$ compared to % CD69+ cells in PBS-injected mice). Although most pronounced on day 1 after infection, up-regulation of CD69 persisted throughout day 7 of infection ($P< 0.05$ compared to PBS-injected mice). Similarly, down regulation of CD62L expression was most pronounced on day 1 after infection, but persisted throughout day 7 ($P<0.05$ compared % CD62L+ cells in PBS-injected mice). Of

note, there was no significant change in the total number of CD4+ and CD8+ T cells during the course of the disease (Figure 2 D).

To assess whether the observed increase in activation markers was associated with effector cytokine production, splenocytes were stained for the presence of intracellular cytokines after stimulation with PMA and Ionomycin, plate-bound anti-CD3 or heat-killed *E. coli*, respectively. No differences in IL-2 or IFNγ staining were observed between *E. coli* and PBS injected mice on either day 1 or day 4 after infection (data not shown).

T cell deficient mice exhibit less severe disease upon E. coli infection than wild-type mice.

Next, we assessed whether absence of T cells had an impact on disease severity. TCRα knock-out mice (TCRα$^{-/-}$), which lack CD4+, CD8+ and NK T cells due to a deletion in the gene for the TCRα-chain, were infected with 1.5×10^8 CFU of *E. coli* and disease severity was compared with that in congenic wild-type mice infected simultaneously with an equal dose. As depicted in Figure 3A, TCRα$^{-/-}$ mice developed only mild and transient weight loss, which was significantly less severe than that observed in wild-type mice ($P<0.01$ after day 1). Similarly, although ruffled fur and hunched appearance were noted in TCRα$^{-/-}$ mice on day 1 after infection, these were less marked than in WT mice ($P<0.05$ for disease scores on day 1 compared to TCRα$^{-/-}$ mice) and resolved over subsequent days. In contrast, WT mice exhibited progressively worsening signs of disease ($P<0.01$ compared to TCRα$^{-/-}$ on days 2-4, Figure 3B). In a separate experiment, *E.coli*-

infected TCR$\alpha^{-/-}$ mice and WT mice were sacrificed on different days after infection, and bacterial growth in blood and liver was assessed. TCR$\alpha^{-/-}$ mice appeared to clear bacteria from the blood sooner than WT mice (cultures negative at day 2 in TCR$\alpha^{-/-}$ mice vs. day 3-4 in WT mice), but cultures from liver remained positive in both groups up to 7 days after infection, with similar CFU counts (Figure 3C). Taken together, these data indicate a pathogenic role for T cells in this *E. coli* model of sepsis, possibly related to a paradoxically delayed clearance of bacteria from the blood stream in the presence of T cells.

Mice deficient in IFNγdevelop less severe disease than wild-type mice and exhibit diminished T cell activation.

We postulated that the pathogenic effect of T cells in this model is most likely mediated through one or more cytokines. The pathogenic effects of IFNγ, a cytokine produced by CD4+, CD8+ as well as NK T cells, have been well described in multiple disease models. To test the hypothesis that IFNγ plays a role in the disease severity of systemic *E. coli* infection in mice, we performed 2 separate sets of experiments. First, IFNγ knock-out (IFNγ$^{-/-}$) mice were infected with 1.5×10^{8} CFU of *E. coli* and disease severity was compared to that in congenic wild-type mice as well as TCR$\alpha^{-/-}$ mice, infected simultaneously with an equal dose. As shown in Figure 4A, weight loss in IFNγ$^{-/-}$ mice was similar to that seen in TCR$\alpha^{-/-}$ mice, and much less severe than in WT mice ($P<0.05$ after day 1). Disease symptoms were also much less pronounced in IFNγ$^{-/-}$ and TCRα^{-}

$^{/-}$ mice compared to WT mice (Figure 4B, P<0.05 for all time points). Although TCR$\alpha^{-/-}$ mice again cleared bacteria from the blood stream by day 2, blood cultures from both WT and IFN$\gamma^{-/-}$ mice stayed positive through day 3 with similar CFU counts for both groups. Bacterial growth in liver was similar for all 3 groups (data not shown). In a separate set of experiments, WT mice were treated with IFNγ-blocking antibodies prior to infection with *E. coli*, and disease severity was compared to *E. coli*-infected, PBS-treated mice. Consistent with the observations in IFN$\gamma^{-/-}$ mice, antibody-depleted mice had less severe weight loss than WT mice (weight loss on day 4, -9.3 ± 6.2% (mean ± standard deviation) vs. -17.1 ± 3.9%, P=0.07) and lower disease scores (disease score on day 4, 1.0 ± 1.0 vs. 3.8 ± 0.5, P<0.01, n=5 mice per group, data not shown.)

We then assessed whether IFN$\gamma^{-/-}$ mice had similar activation of T cells upon *E. coli* infection. Figure 4C shows a quantitative comparison of the results of staining and flow cytometry performed on splenocytes obtained at several time points after infection from *E. coli*-infected IFN$\gamma^{-/-}$ mice and WT mice, respectively. Although IFN$\gamma^{-/-}$ mice exhibited increased expression of CD69 on both CD4+ and CD8+ cells after *E. coli* infection, the percentage of cells expressing this activation marker was significantly reduced when compared to WT mice (mean reduction of 33 – 64%, P<0.05).

Production of cytokines after E. coli infection.

Next, we screened sera obtained from TCR$\alpha^{-/-}$, IFN$\gamma^{-/-}$ and WT mice on day 1 and day 4 after infection with *E. coli* for the presence of a panel of cytokines using multiplex cytokine detection assay. Surprisingly, there was no difference in IFNγ levels between TCR$\alpha^{-/-}$ and WT mice on day 1 (Figure 5A) or day 4 (Figure 5B). There was, however, significantly less IL-6 in the sera of both TCR$\alpha^{-/-}$ and IFN$\gamma^{-/-}$ mice in comparison to WT mice on day 1 (792 ± 318 pg/ml in WT, vs. 328 ± 118 in IFN$\gamma^{-/-}$ mice and 126 ± 88 in TCR$\alpha^{-/-}$, $P<0.05$) as well as day 4 (570 ± 288 pg/ml in WT, vs. 209 ± 69 in IFN$\gamma^{-/-}$ mice and 211 ± 86 in TCR$\alpha^{-/-}$, $P<0.05$). In addition, on day 1 TNFα levels were lower in IFN$\gamma^{-/-}$ compared to WT mice (758 ± 244 pg/ml in IFN$\gamma^{-/-}$ mice vs. 1405 ± 427 in WT mice, $P<0.05$), whereas levels in TCR$\alpha^{-/-}$ were comparable to those in WT mice. TNFα levels on day 4 were similar for all 3 groups.

Discussion

The experiments described here were designed to study the role of T cells in a mouse model of gram-negative sepsis. Surprisingly, activation of a large percentage of T cells occurs very early after i.v. injection of *E. coli*, and absence of T cells is associated with decreased disease severity, indicating a pathogenic role for T cells in this model. Mice lacking IFNγ also develop less severe disease than WT mice upon *E. coli* infection, which appears to be associated with decreased activation of T cells. Amelioration of disease in both IFN$\gamma^{-/-}$ mice and TCR$\alpha^{-/-}$ mice is associated with decreased levels of IL-6.

A role for T cells in sepsis was first suggested by Hotchkiss and colleagues, who demonstrated rapid apoptosis of CD4+ T cells in the murine colonic ligation and puncture (CLP) model of sepsis and showed that prevention of apoptosis resulted in improved outcome [5]. The exact mechanism behind this phenomenon remains unclear. Although we did not study apoptosis in our model, we did not observe depletion of CD4+ T cells in the spleens of *E.coli*-infected mice. This is most likely explained by the difference in models: colonic ligation and puncture results in peritonitis and polymicrobial bacteremia, whereas in our model sepsis does not arise from a nidus of infection and involves one microbe only.

Activation of T cells occurs in response to antigens presented by antigen-presenting cells together with a co-stimulatory signal; this process is thought to take several days. The percentage of antigen-specific T cells for any given antigen is low; therefore only a small percentage of T cells is expected to become activated in response to a microbial infection. Surprisingly, T cell activation was detected within 24 hours after i.v. injection of *E. coli*, and involved a large percentage of T cells (up to 80%). It has been postulated that T cells can respond directly to microbial products such as LPS via direct engagement of TLRs on T cells [6, 7]. *E. coli* infection of mice lacking MyD88, a central adaptor protein for signal transduction of TLRs, resulted in similar early and extensive T cell activation compared to WT mice (data not shown), making this explanation less likely, although signaling through MYD88-independent pathways could still be involved. Early and pronounced up-regulation of CD69 on CD4+ and CD8+ T

cells in response to injection of LPS and other microbial mitogens into mice has been demonstrated previously [8, 9]. In subsequent studies it was shown that the effect of LPS on T cells is mediated by dendritic cells, in part via secretion of IFNαβ, which stimulates T cells directly, and in part by stimulating IFNγ production by NK cells [10]. In concordance with these observations, we demonstrated reduced T cell activation in *E. coli*-infected IFNγ$^{-/-}$ mice. Of note, we did not find evidence for increased IFNγ or IL-2 production by T cells, indicating that the observed activation of T cells may be incomplete and not lead to a full effector phenotype with resulting cytokine secretion.

T cell deficient mice developed significantly less severe disease upon *E. coli* infection than WT mice, indicating that T cells have a detrimental effect on disease outcome. We initially postulated that the pathogenic effect of T cells was mediated by IFNγ, since neutralization of IFNγ has been demonstrated to reduce mortality in mice after LPS injection [11] as well as *E. coli* infection [12]. Indeed, mice lacking IFNγ demonstrated diminished disease severity after *E. coli* infection compared to WT mice. Serum IFNγ levels in WT mice were, however, not different from those in TCRα$^{-/-}$ mice. In addition, we were not able to detect increased IFNγ production by intracellular staining of lymphocytes isolated from spleen or peripheral lymph nodes after restimulation with either plate-bound anti-CD3, PMA and ionomycin, or heat-killed *E. coli* lysates (data not shown). Although this still leaves the possibility of the presence of IFNγ-secreting T cells in other tissues, it seems more likely that, as postulated above, IFNγ is implicated

in the activation and maintenance of T cells rather than in the pathogenic effect of T cells.

Comparison of serum cytokine levels in *E. coli*-infected TCR$\alpha^{-/-}$, IFN$\gamma^{-/-}$ and WT mice revealed a marked difference in IL-6 levels, with much higher levels noted in WT mice both early (day 1) and late (day 4) in the disease course. High serum levels of IL-6 have been demonstrated in human subjects with sepsis, and correlate with an increased risk of mortality [13-16]. It is unclear whether IL-6 is merely a marker of disease severity or whether it is a marker of disease. The results of our studies imply that in the presence of T cells, IL-6 secretion in response to *E. coli* infection is enhanced. This suggests the presence of a positive feedback mechanism that results in ongoing activation of T cells. The precise nature of this postulated feedback mechanism remains to be elucidated. It may be cytokine-mediated, and dependent on T cell activation. A possible candidate is IL-17, which is produced by both CD4+ and CD8+ T cells in response to microbial infection and IL-6 secretion, and augments neutrophil chemoattraction as well as granulopoiesis [17]. Serum IL-17 levels were modestly higher in WT mice than in IFN$\gamma^{-/-}$ or TCR$\alpha^{-/-}$ mice on day 4 after infection; this difference did not reach statistical significance (P=0.08). Although this observation fits with increased IL-6 levels in WT mice, it is at odds with findings in other mouse models, in which IFN$\gamma^{-/-}$ mice were noted to have increased numbers of IL-17 producing T cells [18, 19]. These reports describe findings in models of chronic disease, which may not extrapolate to an acute infectious process. The possible involvement of IL-17 in sepsis deserves further study.

In IFNγ$^{-/-}$ mice, T cell activation after *E. coli* infection was significantly decreased compared to WT mice, but still pronounced compared to PBS-injected mice. In addition, IFNγ$^{-/-}$ mice, but not TCRα$^{-/-}$ mice, had significantly decreased levels of TNFα on day 1 after *E. coli* infection. This suggests that an alternate mechanism may be (in part) responsible for the decreased levels of IL-6 observed in *E. coli*-infected IFNγ$^{-/-}$ mice. Others have shown that IFNγ$^{-/-}$ mice have delayed recruitment and diminished clearance of polymorphonuclear cells in response to an inflammatory stimulus, which is associated with decreased IL-6 levels [20].

Results of our studies do not demonstrate a clear relationship between disease severity and bacterial elimination. Both TCRα$^{-/-}$ and IFNγ$^{-/-}$ mice had markedly less severe disease than WT mice, but only TCRα$^{-/-}$ cleared bacteria faster from the blood stream whereas bacterial load in the liver was not different between the different strains at any of the time points analyzed.

The pathogenesis of sepsis is complex, and our understanding of the immune mechanisms involved remains incomplete. The development of therapeutic modalities for this highly lethal disease is impeded by this lack of insight. The experiments described in this report add to a growing body of evidence that pathogenic immune responses are not limited to the innate immune system. T cells do get activated early after a systemic bacterial infection, and this appears to be detrimental to disease outcome. Our results challenge the idea that adaptive immune responses are slow and protective against infection, and

emphasize the need for further delineation of the interplay between innate and adaptive immune cells and the mediators involved.

Materials and Methods

Mice, bacteria, and infection protocol.

The experiments were approved by The Committee on Animal Research of the University of California, San Francisco.

BALB/c mice and C57BL/6 mice were purchased from Charles River Laboratories (Wilmington, MA). T-cell deficient mice (TCR$\alpha^{-/-}$) on the BALB/c background were kindly provided by Dr. R. Locksley, University of California, San Francisco. IFNγ knock-out mice (IFN$\gamma^{-/-}$), also on the BALB/c background were purchased from Jackson Laboratory (Bar Harbor, ME). Experimental mice were used at 5-7 weeks of age, and age as well as sex matched for each experiment.

A commensal strain of *Escherichia coli (E. coli)* was isolated from feces of mice maintained in the pathogen-free facility of the Laboratory Animal Resource Center at the University of California, San Francisco, CA. Bacteria were cultured in Luria Bertani Broth (LB, Fisher Scientific, Fair Lawn, NJ) at 37^{o}C, harvested at mid-log phase, and washed twice in sterile PBS before injection into the lateral tail vein of experimental mice. Control mice were injected with an equal volume (500 μL) of PBS.

To enumerate the presence of bacteria after infection, serial dilutions of whole blood or organ homogenates were plated on LB plates and incubated at 37°C overnight.

All mice were bred and maintained in the pathogen-free facility of the Laboratory Animal Resource Center at the University of California, San Francisco, CA, in accordance with University guidelines.

After infection with *E. coli*, mice were weighed daily and monitored for signs of disease. A composite disease score was developed to quantify the degree of ruffled fur, hunched appearance and lethargy. Each item was scored 0 (none present), 1 (mild) or 2 (severe), for a maximum total score of 6.

Antibodies

Monoclonal depleting antibodies against CD4 (clone GK1.5), CD8 (YTS-169) and IFNγ (clone R46A2) were a generous gift from Dr. J. Bluestone, University of California, San Francisco. Mice were injected i.p. with 0.5 mg of anti-CD4 antibody, anti-CD8 antibody, or both, 2 days prior to infection with *E. coli*. IFNγ-depleting antibody was injected at 1 mg/dose i.p. 1 day prior to and 1 day after infection with *E. coli.*

FACS analysis

Single cell suspensions were prepared from spleen and peripheral lymph nodes, and stained with anti-CD4 (GK1.5, H129.19, RM4-5), anti-CD8 (53-6.7), anti-CD69 (H1.2F3), anti-CD62L (MEL-14) conjugated to either FITC, PE, PercP or

APC. All antibodies were purchased from BD Pharmingen. Flow cytometric analysis was performed on a FACSCalibur with CellQuest software (BD Biosciences).

Cytokine analysis in serum

Cytokine levels in serum were analyzed with a bead-based assay for simultaneous detection of multiple cytokines (BioPlex Cytokine Assay, Bio-Rad Laboratories) as per the manufacturer's instructions. All samples were tested in duplicate, normal mouse serum served as control.

Statistical analysis

Data were expressed as mean values and standard deviations. Statistical analysis was performed using the Mann-Whitney U test for comparisons between groups.

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Figure Legends

FIGURE 1

WT mice develop a dose dependent systemic illness upon i.v. injection of *E. coli.*

BALB/c mice were injected with either PBS or 1.5×10^7 CFU or 1.5×10^8 CFU of *E. coli* and weight loss (Figure 1A) as well as disease scores (Figure 1B) were recorded on subsequent days. Data points represent means with standard deviations, n=6-9 mice per group. Three mice in the group receiving 1.5×10^8 CFU of *E. coli* died between days 5-6. *P<0.01 comparing 1.5×10^8 CFU group to 1.5×10^7 CFU group **P<0.001 comparing 1.5×10^8 CFU group to 1.5×10^7 CFU group $^{\#\#}$P<0.001 comparing 1.5×10^8 CFU group to PBS group.

FIGURE 2

Activation of T lymphocytes in *E. coli* –infected mice.

A. Splenocytes were isolated at several different time points after injection with PBS or 1.5×10^8 CFU of *E. coli* and analyzed by staining and flow cytometry for expression of CD69 and CD62L. Increased expression of CD69 and decreased expression of CD62L was noted on both the CD4+ gated population (top left panel) and the CD8+ gated population (top right panel) of *E. coli*–infected mice, but not on T cells from PBS-injected mice (bottom panels). FACS blots are representative of typical findings in mice one day after injection. Similar results were obtained when analyzing peripheral lymph node cells (data not shown).

B. Quantification of the percentage of CD69+ cells of total CD4+ (left panel) and CD8+ T cells (right panel) from splenocytes. Data are expressed as means with standard deviations, n=4 mice per group. *P<0.05 comparing *E.coli*-infected to PBS-injected mice.

C. Quantification of the percentage of CD69+CD62^{lo} cells of total CD4+ (left panel) and CD8+ T cells (right panel) from splenocytes. Data are expressed as means with standard deviations, n=4 mice per group. *P<0.05 comparing *E.coli*-infected to PBS-injected mice.

D. Quantification of total number of CD4+ cells (left panel) and CD8+ cells (right panel) from splenocytes. Data are expressed as means with standard deviations, n=4 mice per group.

FIGURE 3

T cell deficient mice are relatively resistant to infection with *E. coli*.

A-B. TCR$\alpha^{-/-}$ mice and age-matched congenic WT mice were inoculated with 1.5 × 10^8 CFU of *E. coli* and weight loss (Figure 3A) as well as disease scores (Figure 3B) were recorded on subsequent days. Data points represent means with standard deviations, n=5-6 mice per group. # P<0.05, *P<0.01 as compared to WT mice.

C. In a separate experiment, *E. coli*-infected TCR$\alpha^{-/-}$ and WT mice were sacrificed on different days after infection, and bacterial growth in blood and liver was quantified (Figure 3C). Data points represent means with standard deviations, n=3-4 mice per group per time point.

FIGURE 4

Interferon-γ deficient mice are relatively resistant to infection with *E. coli* and exhibit decreased T cell activation.

A-B. Age-matched IFNγ$^{-/-}$, TCRα$^{-/-}$ mice and WT mice (all on the BALB/c background) were inoculated with 1.5 × 10^{8} CFU of *E. coli* and weight loss (Figure 4A) as well as disease scores (Figure 4B) were recorded on subsequent days. Data points represent means with standard deviations, n=4-6 mice per group, *P<0.05 as compared to WT mice.

C. IFNγ$^{-/-}$ mice and congenic, age matched WT mice were inoculated with 1.5 × 10^{8} CFU of *E. coli* and splenocytes were isolated on subsequent days and analyzed for expression of the activation marker CD69 by staining and flow cytometry. Percentage of CD69+ cells among the CD4+ gated population (left panel) and the CD8+ gated population (right panel) were calculated. Data points represent means with standard deviations, n=3 mice per group. *P<0.05 comparing IFNγ$^{-/-}$ to WT mice.

FIGURE 5

Serum cytokine levels in *E. coli*-infected mice.

Serum obtained from *E. coli* –infected IFNγ$^{-/-}$, TCRα$^{-/-}$ and congenic WT mice on day 1 (Figure 5A) and day 4 (Figure 5B) after infection were analyzed for cytokine expression by multiplex detection assay as described in methods and

materials. All samples were tested in duplicate; circles represent the mean value for each animal tested, lines represent the mean value per group. *P<0.05

FIGURE 1

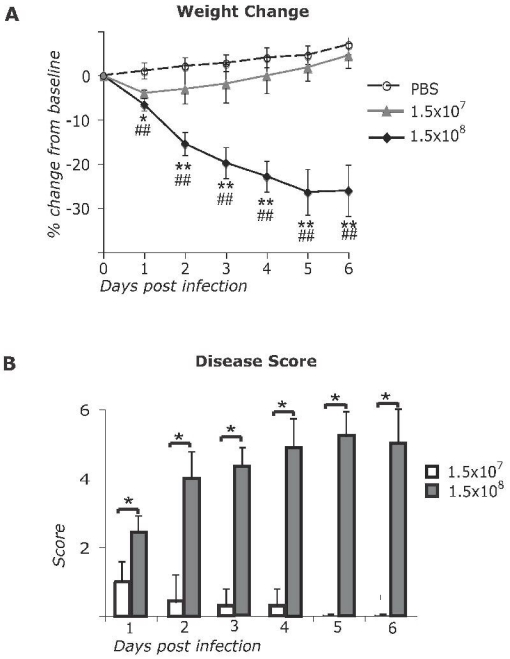

A
Weight Change
% change from baseline
0
-10
-20
-30
Days post infection
PBS
1.5x10^7
1.5x10^8
B
Disease Score
Score
Days post infection
1.5x10^7
1.5x10^8

FIGURE 2

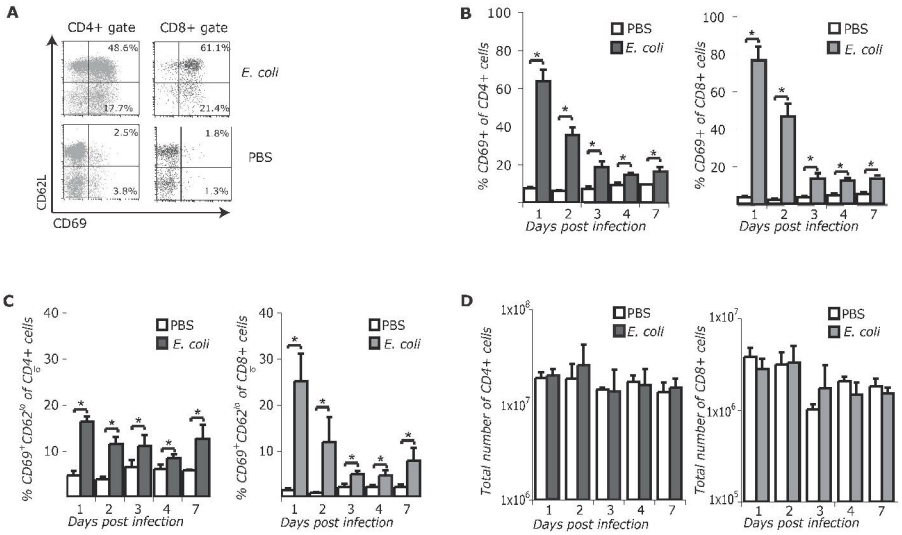


FIGURE 3

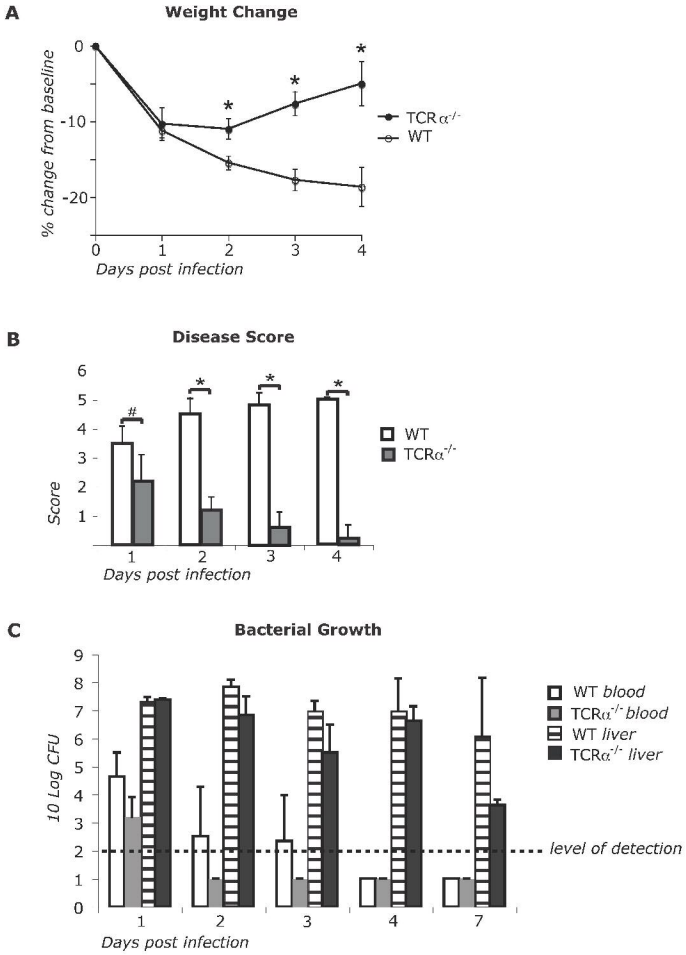

A
Weight Change
% change from baseline
0
-10
-20
0 1 2 3 4
Days post infection
TCR α-/-
WT
B
Disease Score
Score
1 2 3 4 5 6
1 2 3 4
Days post infection
WT
TCRα-/-
C
Bacterial Growth
10 Log CFU
0 1 2 3 4 5 6 7 8 9
1 2 3 4 7
Days post infection
WT blood
TCRα-/- blood
WT liver
TCRα-/- liver
level of detection

FIGURE 4

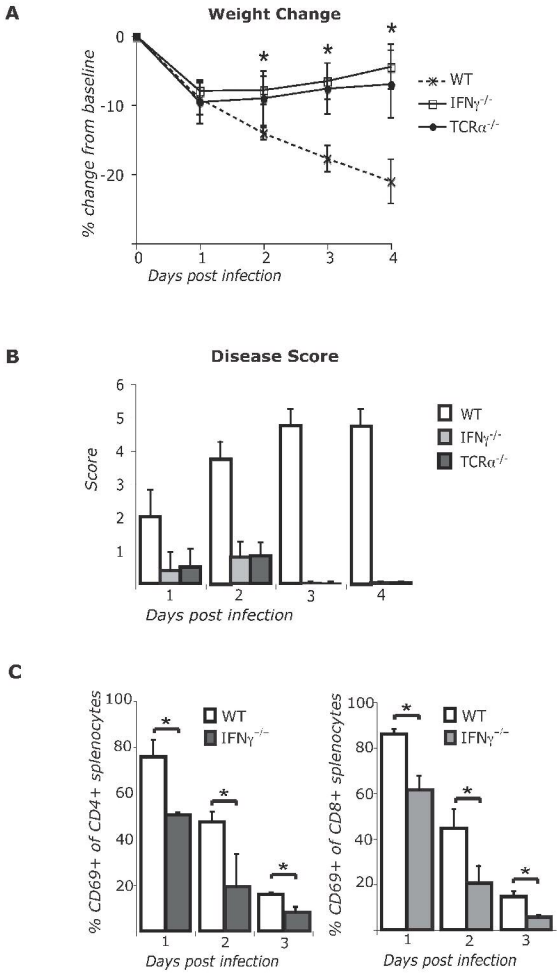
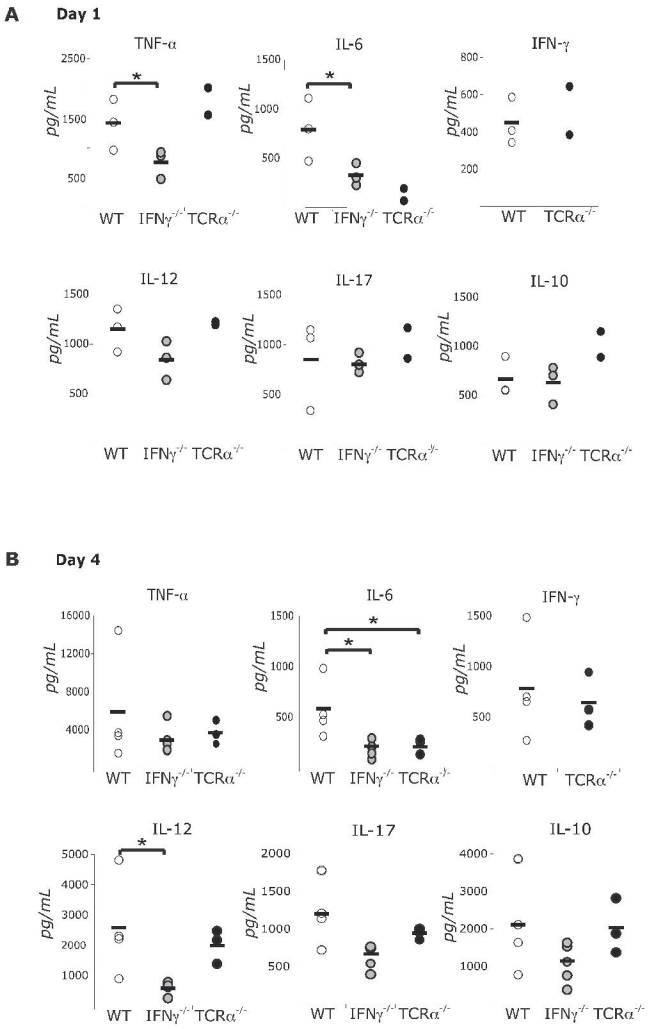

A
Weight Change
% change from baseline
Days post infection
WT
IFNγ-/-
TCRα-/-
B
Disease Score
Score
Days post infection
WT
IFNγ-/-
TCRα-/-
C
% CD69+ of CD4+ splenocytes
% CD69+ of CD8+ splenocytes
Days post infection
Days post infection
WT
IFNγ-/-

FIGURE 5


A
Day 1
TNF-α
IL-6
IFN-γ
IL-12
IL-17
IL-10
pg/mL
WT
IFNγ-/- TCRα-/-
TCRα-/-
B
Day 4
TNF-α
IL-6
IFN-γ
IL-12
IL-17
IL-10
pg/mL
WT
IFNγ-/- TCRα-/-
TCRα-/-