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DEFICIENCY OF $\gamma\delta$ T-LYMPHOCYTES CONTRIBUTES TO MORTALITY AND IMMUNOSUPPRESSION IN SEPSIS

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Abstract

Studies have indicated that $\gamma\delta$ T-lymphocytes play an important role in the regulation of immune function and the clearance of intracellular pathogens. We have recently reported that intraepithelial lymphocytes (IEL), which is rich in $\gamma\delta$ T-cells, within the small intestine illustrated a significant increase in apoptosis and immune dysfunction in mice subjected to sepsis. However, the contribution of $\gamma\delta$ T-cells to the host response to polymicrobial sepsis remains unclear. In this study, we initially observed that following sepsis induced by cecal ligation and puncture (CLP) there was an increase in small intestinal IEL CD8⁺- $\gamma\delta$ ⁺ T-cells in control $\gamma\delta$ +/+ mice. Importantly, we subsequently found an increased early mortality in mice lacking $\gamma\delta$ T-cells ($\gamma\delta$ -/-) after sepsis. This was associated with a decrease in plasma TNF- α , IL-6 and IL-12 levels in $\gamma\delta$ -/- mice compared to $\gamma\delta$ +/+ mice after sepsis. In addition, even though *in vitro* LPS-stimulated peritoneal macrophages showed a reduction in IL-6 and IL-12 release after CLP, these cytokines were less suppressed in macrophages isolated from $\gamma\delta$ -/- mice. Alternatively, IL-10 release was not different between septic $\gamma\delta$ +/+ and $\gamma\delta$ -/- mice. While Th1 cytokine release by anti-CD3-stimulated splenocytes was significantly depressed in septic $\gamma\delta$ +/+ mice, there was no such depression in $\gamma\delta$ -/- mice. However, $\gamma\delta$ T-cell deficiency had no effect on Th2 cytokine release. These findings suggest that $\gamma\delta$ T-cells may play a critical role in regulating the host immune response and survival to sepsis, in part by alteration of the level of IEL CD8⁺- $\gamma\delta$ ⁺ T-cells and through the development of Th1 response.

Keywords

Th1 cytokines; intraepithelial lymphocytes; mice

INTRODUCTION

Although there have been major technological advances in the treatment of traumatic/surgical injury, post-operative complications such as sepsis, remain one of the most common causes of morbidity and mortality in the intensive care unit (8). Studies have indicated that there is a marked suppression of immune function during sepsis (25,29), which may contribute to the development of subsequent multiple organ failure. The mechanism behind the induction of immunosuppression in sepsis is only beginning to be understood. In this respect, accumulative evidence has consistently suggested the existence of active suppressor/regulator lymphoid populations such as CD4⁺ Th-2, T-regulatory, Th3, NK-T, CD8⁺ as well as $\gamma\delta$ T-cells, which might be responsible for mediating aspects of this immune dysfunction(30). Our as well as other laboratories have previously demonstrated that the development of Th2-cells, which

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exhibit an immune suppressive phenotype, occurs in response to septic challenge (3,24). However, studies revealing the role of $\gamma\delta$ T-lymphocytes in trauma/sepsis are still limited.

Murine $\gamma\delta$ T-lymphocytes comprise about 5–10 % of the total T-cell population, however, they are especially predominant in mucosal surface such as epithelial layers of the gut, the tongue, the lung and the female reproductive tract(20). It is becoming clear that unlike $\alpha\beta$ T-cells, which recognize processed peptide antigens/MHC complexes, $\gamma\delta$ T-cells appear to recognize a variety of nascent proteins without antigen processing (11). Therefore, pathogens, damaged tissue and/or T- and B-cells can be recognized directly and cellular immunity can be initiated without antigen-presenting cells or prior antigen degradation(28). Along with their unique features in antigen recognition and localization in the mucosa, $\gamma\delta$ T-cells have been shown to produce a variety of cytokines upon stimulation. However, while the important roles of $\alpha\beta$ T-cells in immune function are well studied, our comprehension of the biological functions of $\gamma\delta$ T-cells remains incomplete. Allison and Havran(1) proposed that $\gamma\delta$ T-cells may be responsible for the first line of defense. This concept is supported by the increase/accumulation of $\gamma\delta$ T-cells earlier before $\alpha\beta$ T-cell responses, in bacterial and viral infections (18,35). An increasing number of studies have been documented and suggest that $\gamma\delta$ T-cells may play a role in regulation of immune function(13,28) and may also down-regulate the immune response during bacterial infection by producing cytokines (26). Several studies have shown that $\gamma\delta$ T-cells are critical for mouse survival following burn injury(38), *Klebsiella pneumonia* (33), and pro-inflammatory cytokine development (33,38). Furthermore, recent clinical studies have shown that circulating $\gamma\delta$ T-cell count was significantly lower in septic patients compared with normal healthy donors (31,45), which suggests that $\gamma\delta$ T-cells may play an important role in the inflammatory response after sepsis. Nonetheless, the precise role of $\gamma\delta$ T-cells in sepsis-induced immune depression remains unclear.

Previous studies in our laboratory using cecal ligation and puncture, to produce a polymicrobial septic challenge that is thought to more closely approximate clinical sepsis (14), indicated that there is dysfunction in the intraepithelial lymphoid compartment of the small intestine, which is a site rich in $\gamma\delta$ T-cells. However, the contribution of $\gamma\delta$ T-cells was not directly examined in that study(12). We, therefore, wished to test the hypothesis that $\gamma\delta$ T-cells play a role in the regulating immune response in polymicrobial septic mouse. In the current study, we investigated the effect of $\gamma\delta$ T-cell deficiency on mouse overall survival and possible suppression/regulation in immune function during the course of polymicrobial sepsis.

MATERIALS AND METHODS

Cecal Ligation and Puncture (CLP)

Polymicrobial sepsis was induced in mice using the model of cecal ligation and puncture described by our laboratory(4). Male inbred C57BL/6J (background control, $\gamma\delta$ +/+) and C57BL/6J^{trdMom} ($\gamma\delta$ -/-) (Jackson Laboratory, Bar Harbor, ME) mice, 7–10 weeks of age, were lightly anesthetized with Metofane (Methoxyflurane Pitman-Moore, Mundelein, IL), shaved at the abdomen and scrubbed with betadine. A midline incision (1.5–2.0 cm) was made below the diaphragm to expose the cecum. The cecum was ligated and punctured twice with a 22-gauge needle and gently compressed to extrude a small amount of cecal contents through the punctured holes. The cecum was returned to the abdomen and the incision was sutured in layers. The animals then were resuscitated with 0.8 ml of lactated Ringer's solution by subcutaneous injection. For sham-controls, the cecum was extracted but neither ligated nor punctured. All procedures were carried out according to the National Institute of Health Guidelines on Laboratory Animals and approved by the Animal Welfare Committee of Rhode Island Hospital.

For the survival study, $\gamma\delta$ $+/+$ and $\gamma\delta$ $-/-$ mice were subjected to CLP procedure and monitored for their mortality over a 10-day period.

CD8⁺ IEL Isolation and Flow Cytometric Analysis

Animals were sacrificed at 4 or 24h after CLP or sham-CLP by Metofane overdose and the small intestine was immediately removed and washed thoroughly with cold HBSS (Ca and Mg-free, Gibco-BRL). The Peyer's patches, fat and mesentery were dissected out and the gut was slit open and cut into segments (0.5–1 cm). The gut segments were incubated for 90 min at 37° C in three changes of 25 ml HBSS containing 1 mM dithiothreitol and 1 mM EDTA (Sigma, St. Louis, MO) with continuous shaking in a water bath. The cell suspension from three incubations were pooled, centrifuged, resuspended and filtered by passing them through a loosely packed nylon wool column at room temperature to remove mucus, tissue debris and dead cells (12). The cells were then washed and further purified using magnetic cell sorting column according to manufactory's instructions (Miltenyi Biotec, Inc., Auburn, CA). In brief, cells were incubated with anti-CD8 microbeads (in the dark at 4–8° C for 15 min) and magnetically separated with the columns. The CD8-enriched cell suspensions were then stained with antibodies against $\alpha\beta$ (clone H57–597, hamster IgG2) or $\gamma\delta$ (clone GL3, hamster IgG2) T-cell receptors from BD-Pharmingen Inc. (San Diego, CA). Two-color flow cytometric analysis was carried out according to methods previously described by our laboratory (12). Analysis was performed using the PC-lysis software (BD-Pharmingen).

Preparations of Splenocytes

Splenocytes were isolated 24h post-surgery using the method described by Meldrum et al (32). In brief, spleens were gently glass ground and erythrocytes hypotonical lysed and the isolated splenocytes were then washed and counted. Splenocyte cultures were incubated in plastic tissue culture plates for 2h to deplete adherent cells. The non-adherent splenic lymphocytes were collected, washed and then resuspended in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (Gibco-BRL, Grand Island, NY) to 1×10^6 cells per mL. The viability of the recovered cells was determined by trypan blue exclusion and was found to be >95%.

Preparations of Peritoneal Macrophages

Resident peritoneal macrophages were obtained 24h after surgery by lavage as previously described (5). Peritoneal macrophages were harvested by lavage of the peritoneal cavity with 2×5 mL of cold Dulbecco's modified Eagle's medium (DMEM Gibco-BRL). The cells were centrifuged (800xg at 4° C for 15 min) and resuspended in fresh DMEM at 1×10^6 cells/mL and then plated onto plastic tissue culture plates and incubated at 37° C for 2h. The nonadherent cells were removed by repeated washing three times with fresh DMEM. This protocol provided adherent cells that were greater than 95% positive by nonspecific esterase staining and that exhibited typical macrophage morphology.

Stimulation and Assessment of Cytokine Release

Splenocytes were stimulated *in vitro* with or without monoclonal rat anti-mouse CD3 antibody (15 μ g per mL in PBS, coated overnight at 4° C in tissue culture plates) for 48h at 37° C (5% CO₂ 95% humidity). Adherent macrophage monolayers were stimulated with or without 10 μ g LPS/mL of DMEM medium supplemented with 10% fetal calf serum for 24h (37° C, 5% CO₂). At the end of the incubation period, the cultured supernatants were collected and stored at –70° C until analysis.

Th1 (IL-2 and IFN- γ) or Th2 (IL-10) cytokines produced from splenocytes upon plate-bound anti-CD3 stimulation and IL-6, IL-12 and IL-10 produced from macrophages upon LPS

stimulation were measured in culture supernatants by the “sandwich enzyme-linked immunosorbent assay (ELISA)” technique as previously described (3) with monoclonal antibody pairs and the appropriate mouse cytokine standards obtained from BD-Pharmingen, Inc.

Plasma Levels of Pro-inflammatory Cytokines

Blood was collected by cardiac puncture in a syringe containing 2 units of heparin, transferred to a microtube, and centrifuged immediately at $10,000 \times g$ for 10 minutes at 4°C . Plasma samples were stored at -70°C until analyzed. The levels of TNF- α , IL-6 and IL-12 were measured by ELISA (BD-Pharmingen).

Presentation of Data and Statistical Analysis

The data are presented as mean $\pm$ SEM for each group. Differences in percentile data (i.e., percentage of apoptosis positive and phenotypic positive) and cytokine levels were considered to be significant if $P < 0.05$, as determined by the Mann-Whitney U-test. The survival data were compared using the Fisher exact and Kaplan-Meier/Log-rank test, and considered being significant at $P < 0.05$.

RESULTS

Sepsis induces a concomitant increase in the percentage of CD8 $^{+}$ $\gamma\delta^{+}$ and a decrease in CD8 $^{+}$ $\alpha\beta^{+}$ cells in small intestinal lymphoid compartment of the gut

IEL phenotypic distribution was characterized by staining the cells with anti- $\alpha\beta$ or- $\gamma\delta$ T-cell receptors and anti-CD8. In control $\gamma\delta$ $+/+$ mice, CD8 $^{+}$ $\alpha\beta^{+}$ and CD8 $^{+}$ $\gamma\delta^{+}$ are about equally distributed in the small intestine of sham animals. Figure 1 demonstrates that the percentage of CD8 $^{+}$ $\alpha\beta^{+}$ and CD8 $^{+}$ $\gamma\delta^{+}$ cells of total CD8 positive cells in IEL isolated from sham or CLP mice. While total CD8 positive (data not shown) and CD8 $^{+}$ $\alpha\beta^{+}$ IELs decreased in CLP mice, there was a significant increase in CD8 $^{+}$ $\gamma\delta^{+}$ cells at 4h following the onset of sepsis. Although not statistically significant, the percentage of CD8 $^{+}$ $\gamma\delta^{+}$ cells at 24h was still higher in septic mice.

Mice lacking of $\gamma\delta$ T cells succumb more rapidly following the initial onset of polymicrobial sepsis

To determine whether $\gamma\delta$ T cell deficiency could affect the animals' ability to survive septic challenge, C57BL/6J (background control, $\gamma\delta$ $+/+$) and C57BL/6J terd^{Mom} ($\gamma\delta$ $-/-$) mice were subjected to CLP and survival was monitored for 10 days. $\gamma\delta$ $-/-$ mice showed a significantly increased mortality rate early following CLP (day 1, $P < 0.05$ Fisher exact test) (Fig. 2). However, while a difference of $\sim 20\%$ persisted over time (>2 days), this was no longer statistically different based on Log-rank/Kaplan-Meier type comparison, which assesses cumulative change over time using typically a much larger n/group . Nevertheless, this does not reduce the significance of the changes seen early after CLP.

$\gamma\delta$ T cell deficiency suppressed systemic levels of pro-inflammatory cytokine release after CLP

At both 4 and 24h after CLP, plasma levels of pro-inflammatory cytokines such as: TNF- α , IL-6 and IL-12 (Fig. 3 A, B and C) were significantly elevated compared with the sham control $\gamma\delta$ $+/+$ mice. Alternatively, $\gamma\delta$ $-/-$ mice did not respond to septic challenge as vigorously as $\gamma\delta$ $+/+$ mice. At 4h, although cytokine levels in $\gamma\delta$ $-/-$ septic mice were significantly elevated compared with their shams, they were markedly lower than $\gamma\delta$ $+/+$ septic mice. By 24h after CLP, the production of these cytokines in $\gamma\delta$ $-/-$ septic mice was essentially absent.

$\gamma\delta$ T cell deficiency ablates the suppression of Th1 but not Th2 cytokine release in anti-CD3 stimulated splenocytes after CLP

While Th1 cytokine (IL-2 and IFN- γ) release by splenocytes in response to anti-CD3 stimulation in $\gamma\delta$ +/+ mice was decreased compared with their shams 24h after sepsis, no such suppression was seen in $\gamma\delta$ -/- mice (Fig. 4A-B). However, though an increase in Th2 cytokine, such as IL-10 release after CLP was observed, this was not attenuated in the $\gamma\delta$ -/- mouse cells, there were no changes between the two mouse strains (Fig. 4C). No cytokines were detected in the absence of stimulation (data not shown).

Effect of $\gamma\delta$ T cell deficiency on IL-6, IL-12 and IL-10 release in LPS-stimulated peritoneal macrophages after CLP

Figure 5 illustrates that peritoneal macrophages harvested from septic $\gamma\delta$ +/+ mice show a significant decrease in IL-6 (Fig. 5A) and IL-12 (Fig. 5B) release in response to LPS compared to their shams. Deficiency of $\gamma\delta$ T-cells also suppressed IL-6 release by peritoneal macrophages from septic $\gamma\delta$ -/- mice, however, the degree of suppression was significantly less than that seen in the control mice. IL-12 release in peritoneal macrophages was not suppressed in $\gamma\delta$ -/- mice after CLP. Alternatively, $\gamma\delta$ T-cells had no effect on increased IL-10 release in peritoneal macrophages after sepsis (Fig. 5C). It should be noted that without LPS stimulation a significant increase in IL-6 and IL-12 release was observed in control $\gamma\delta$ +/+ mouse peritoneal macrophages after sepsis, however, there was no or low cytokine release in septic $\gamma\delta$ -/- mice. In addition, IL-10 release was not detectable without LPS stimulation in peritoneal macrophages from both mouse strains.

DISCUSSION

The fact that $\gamma\delta$ T-cell deficiency contributes to the high mortality rate seen in many different models of bacterial infection suggests that $\gamma\delta$ T-cells are critical to the overall protection of host against pathogenic insults (22,39,41). Studies have shown that loss of immune (T-, B-cells and monocytes) and/or non-immune (epithelial, myocytes, endothelial and hepatocytes) cells through the apoptotic process may contribute to the immunosuppression seen in sepsis, which may lead to the subsequent multiple organ failure and mortality(2,10,25). In this study, we have reported that $\gamma\delta$ T-cell deficient ($\gamma\delta$ -/-) mice succumb more rapidly earlier when compared with the background control ($\gamma\delta$ +/+) mice following the induction of sepsis by cecal ligation and puncture (CLP). One possible explanation for the higher mortality seen in $\gamma\delta$ -/- mice during early stage of polymicrobial sepsis could be related to the deficit of $\gamma\delta$ T-cells in the small intestinal IEL compartment, as we have observed an increase in IEL $\gamma\delta$ T-cells taken from control $\gamma\delta$ +/+ mice. Increased numbers of $\gamma\delta$ T-cells have been shown to be associated with a variety of infectious conditions (9). Studies have indicated that an accumulation of $\gamma\delta$ T-cells was found in the lung of mice with sepsis (23) and pulmonary bacterial infection(44). Nonetheless, $\gamma\delta$ T-cells seem to have a complex role in host defense as the depletion of these cells has been shown to lead to not only changes in Th1 and Th2 type responses but can be either detrimental or protective to host survival/infection depending on specific pathogen nature and the stage of infection(28).

Another explanation for high mortality in septic mice could be related to the apparent inability of these mice to mount an adequate pro-inflammatory (innate) response. In this respect, one of the most striking differences between the $\gamma\delta$ +/+ and $\gamma\delta$ -/- mice was that little or no increase in the systemic pro-inflammatory cytokines levels (TNF- α , IL-6 and IL-12) were detected in $\gamma\delta$ deficient mice in response to sepsis. The current paradigm for the pathophysiology of shock/sepsis is that organ injury results from uncontrolled pro-inflammatory response (19). Pro-inflammatory cytokines such as TNF- α , IL-1 β and IL-6, have been suggested to be responsible for the initiation of cell and organ dysfunctions associated with sepsis and multiple organ failure

(7). This based primarily on the observation made in that when high bacterial toxin and/or mono-specific microbe infusion or high concentrations of the given cytokine (e.g. TNF, IL-1 β , etc.) in various animal models, that shock and death were the common result. However, when the dosage of these pro-inflammatory agents has been titrated back towards levels actually encountered in septic animals/patients or where the model used produces a somewhat more comparable state to that of the septic patient (e.g., CLP used here) (14), the extent of pro-inflammation observed is substantially lower than that seen in models of toxic shock. Further, a number of investigators have demonstrated that ablating key members of this pro-inflammatory response to septic challenge can actual result in significant harm to the animal (6,17,21) suggesting that $\gamma\delta$ T-cells maybe essential in controlling an inflammatory response. Thus, the depletion of these cells may lead to dysregulation and/or mortality. The failure of anti-inflammatory therapeutic trials for sepsis also suggests that the process by which septic patients develop multiple organ failure is far more complex than that produced in simple models of lethal toxic/bacterimic shock. Therefore, the early pro-inflammatory mediator response may reflect the animals attempt to contain respond to the developing infection in CLP and hence be an important component for survival.

The contribution of $\alpha\beta$ TCR+ T-cells let alone $\gamma\delta$ TCR+ T-cells to the development of the innate/pro-inflammatory response is poorly understood, $\alpha\beta$ T-cells are for the most part thought to play a critical role in the acquired/adaptive response to infection and not innate immunity (27,43). The impact of $\gamma\delta$ T-cells on innate and/or adaptive immunity during infection is even less well understood. However, a number of recent studies appear to suggest that $\gamma\delta$ T-cells may play a critical role in the cross talk between T-cells in the development of an adaptive response and macrophages during the induction of an innate immunity. Macrophages from $\gamma\delta$ -/- mice are defective in TNF- α production, suggesting that a mechanism exists for $\gamma\delta$ T-cells where by cellular components that contribute to the regulation of the innate responsiveness (34). Moore *et al.* (15) reported that $\gamma\delta$ T-cell deficiency impaired early expression of TNF- α and IFN- γ mRNA, which was associated with increasing susceptibility to *Klebsiella pneumoniae* infection. $\gamma\delta$ T-cell depletion has been shown to down-regulate chemokine and chemokine receptor expression in a model of experimental autoimmune encephalomyelitis (36). Studies have also suggested that $\gamma\delta$ T-cells can function as a potent source of cytokines themselves that may stimulate or attract other cells into a site of inflammation(37). In this regard, our results further support the suggestion that $\gamma\delta$ T-cells may play a critical regulatory role in development of a competent innate/pro-inflammatory response. However, the precise nature of the interaction between these cells is still unclear.

Previous studies from many laboratories including our own indicate that Th1 cytokine release in anti-CD3 stimulated splenocytes is suppressed with enhancement of Th2 cytokine release following the onset of sepsis (2). However, little is known about the mechanism responsible for these changes. Here we have observed that Th1 cytokine release in splenocytes from septic $\gamma\delta$ -/- mice was not suppressed as is typically seen in $\gamma\delta$ +/+ mice. This suggests at least two things about the immune response seen in the $\gamma\delta$ -/- animals. First, the restoration of splenocyte IL-2/IFN- γ response suggests the $\gamma\delta$ T-cells may contribute directly or indirectly to the developing Th1-cell dysfunction. Secondly, the development of this late immune dysfunction may not be a critical component contributing to mortality in the $\gamma\delta$ T-cell deficient animal. In this respect, others and we have previously reported that anti-inflammatory agents, such as IL-10, which can modulate the induction of suppression of the cell mediated/lymphoid immune response, can also have marked effects on the innate/pro-inflammatory responsiveness (16, 40). We found that when mice were administered a neutralizing antibody to IL-10 immediately following induction of CLP, a time point at the beginning of the pro-inflammatory phase in this sepsis model, that survival of these animals was markedly reduced early after CLP (42). However, when the antibody was given at 12 h post CLP, a period just outside the majority of the pro-inflammatory response but prior to marked splenic immune suppression, survival was

not only improved but Th1 responsiveness was preserved. This demonstrates that the susceptibility to septic morbidity and mortality can be differentially affected by the nature phase of the host response that may predominate at the time of treatment, i.e., early defects in innate response versus the late development of cell-mediated changes. Thus, we suggest that alternations in the innate response more likely may be effected by this deficiency in $\gamma\delta$ T-cells, while the change in cell-mediated (Th1) responsiveness is a secondary aspect. However, further studies are needed to delineate the effect of these T-cells on innate as well as cell-mediated immune responsiveness.

In summary, our findings indicated that deficiency in $\gamma\delta$ T-cells may induce early mortality in sepsis. An increase in $\gamma\delta$ T-cells in the small intestinal IELs was observed after sepsis. The extensive decrease in the systemic level of the pro-inflammatory cytokine response along with the lack of changes in Th1 cytokine release observed in splenic T-cells and the attenuation of pro-inflammatory cytokine release by peritoneal macrophages, in septic $\gamma\delta^{-/-}$ mice appear to implicate the $\gamma\delta$ T-cells' role in communication between adaptive (cell-mediated) responsiveness and innate response mediated by macrophage, granulocyte, epithelial cells, etc. Thus, while accounting for only a small fraction of all mature T-cells, the $\gamma\delta$ T-cells would appear to play a critical role in maintaining an innate response, that is sufficient to ward off the lethal effects of polymicrobial septic challenge.

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Fig. 1.

Percentage of cells positively stained with anti-CD8, αβ or γδ T-cell receptors in IELs isolated from C57BL/6J (γδ +/+) mice 4 or 24h after CLP. A significant increase in the percentage of CD8+γδ+ cells was observed at 4 and 24h, while CD8+αβ+ cell percentage was decreased after sepsis. Significance indicated by * at p<0.05 vs. sham, t-test, Mean ± SEM n=6 mice/group.

Fig. 2. Percent survival of C57BL/6J (γδ +/+) and C57BL/6J^{Tcrd^{tm1Mb/m}} (γδ -/-) mice following sepsis induced by CLP. Survival was recorded for 10 days. Significance indicated by * at p<0.05 vs. C57BL/6J, Fisher Exact test n=19–20 mice/group.

Fig. 3. Plasma levels of cytokines in sham and septic C57BL/6J ($\gamma\delta$ +/+) and C57BL/6J $\gamma\delta$ ^{-/-} mice. TNF- α (A), IL-6 (B) and IL-12 (C) levels were significantly less in septic $\gamma\delta$ -/- mice compared to septic $\gamma\delta$ +/+ mice at both 4 and 24h after sepsis. Significance indicated by * at $p < 0.05$ vs. sham and # vs. $\gamma\delta$ +/+ mice, Mann-Whitney U test, Mean $\pm$ SEM $n = 6-7$ mice/group.

Fig. 4. IL-2 (A), IFN- γ (B) and IL-10 (C) release in splenocytes from septic or sham C57BL/6J ($\gamma\delta$ +/+) and C57BL/6J^{trdMom} ($\gamma\delta$ -/-) mice. Splenocytes were isolated 24h after surgery and stimulated with immobilized anti-CD3 for 48h. Supernatants were then collected for cytokine determination by ELISA. Significance indicated by * at p<0.05 vs. sham, Mann-Whitney U test, Mean $\pm$ SEM n=6–7 mice/group.

Fig. 5. IL-6 (A), IL-12 (B) and IL-10 (C) release in peritoneal macrophages from sham or septic C57BL/6J ($\gamma\delta$ +/+) and C57BL/6J^{trdMom} ($\gamma\delta$ -/-) mice. Macrophages were isolated 24h after surgery and stimulated with LPS for 24h. Supernatants were then collected for cytokine determination by ELISA. Significance indicated by * at $p < 0.05$ vs. sham and # vs. $\gamma\delta$ +/+ mice, Mann-Whitney U test, Mean $\pm$ SEM $n = 6-7$ mice/group. ND: not detectable.