Published in final edited form as:

Inflammation. 2018 February ; 41(1): 328–336. doi:10.1007/s10753-017-0690-6.

Poly(I:C) priming exacerbates cecal ligation and puncture-induced polymicrobial sepsis in mice

Deepika Sharma, Ankit Malik, Nandakumar Packiriswamy, Michael D. Steury, and Narayanan Parameswaran*

Department of Physiology, Michigan State University, East Lansing, MI 48824, USA

Abstract

Sepsis continues to be a major healthcare issue with one of the highest mortality rates in the intensive care units. Toll-like receptors are pattern recognition receptors that are intricately involved in the pathogenesis of sepsis. TLR3 is a major receptor for double-stranded RNA and is largely associated with immunity to viral infection. In this study, we examined the role of TLR3 priming in the immunopathology of sepsis using cecal-ligation and puncture (CLP) model of sepsis in mice. Mice injected with vehicle or poly(I:C) were subjected to sham or CLP surgery and various parameters of sepsis, including mortality, inflammation and bacterial clearance were assessed. Poly(I:C) pretreatment significantly enhanced mortality in mice subjected to CLP. Consistent with this, inflammatory cytokines including TNF α , IL-12p40, IFN γ and MCP-1 were enhanced both systemically and locally in the poly(I:C) treated group compared to the vehicle control. In addition, bacterial load was significantly higher in the poly(I:C) treated septic mice. These changes were associated with reduced macrophage activation (but not neutrophils) in the peritoneal cavity of poly(I:C) pre-treated mice compared to vehicle pretreatment. Together our results demonstrate that poly(I:C) priming in sepsis is likely to be detrimental to the host due to effects on systemic inflammatory cytokines and bacterial clearance.

Introduction

Toll-like receptors are pattern recognition receptors that recognize pathogen associated molecular patterns (PAMPs) or damage associated molecular patterns (DAMPs) that mark microbial infection or sterile damage. By regulating some of the key inflammatory pathways, TLRs are now recognized as critical players in the pathogenesis of various diseases. Among these, polymicrobial sepsis exemplifies the role of TLRs in various pathological events [22]. While it is clear that TLRs are integral in pathogenesis of sepsis, their precise role in various integrative processes during sepsis has become more complex. In this regard, TLRs are important not only in the induction of inflammatory responses but also in the resolution process that provides a critical balance between microbial clearance and tissue repair without undue damage to the host tissue [18, 22]. Thus, a balanced inflammatory response is dependent on many factors including the inflammatory status of the host. This is exemplified during endotoxin tolerance where prior exposure to

*Corresponding author: Narayanan Parameswaran BVSc., Ph.D., 567 Wilson Road, #2201 Biomedical Physical Sci. Bldg., Department of Physiology, East Lansing, MI 48824, USA, narap@msu.edu.

lipopolysaccharide (LPS) renders the host tolerized to subsequent LPS stimulation (i.e. endotoxin tolerance). This has been shown to be beneficial in the context of polymicrobial sepsis, where prior exposure to endotoxin, renders the mice less susceptible to cecal-ligation and puncture-induced bacterial load, organ injury and mortality. Similar protection has been observed with prior treatments with TLR2 and TLR9 ligands [26, 25, 24, 21, 17, 8, 7]. However, whether prior exposure of TLR3 ligand modulates CLP-induced sepsis pathogenesis is not well known [3].

Even though direct evidence for cross-tolerance in response to TLR3 is lacking in the CLP model, previous studies have shown positive effects of poly(I:C) priming on bacterial phagocytosis. Poly(I:C) priming upregulates bacterial phagocytosis but downregulates apoptotic neutrophil uptake through IRF3 (type I IFN) and NF κ B pathways (TNF α) respectively [5]. Another study however showed TLR3 as poor inducers of bacterial uptake as compared to TLR9 which was the strongest inducer of bacterial phagocytosis [6].

Poly(I:C) is often used to bolster a Th1 or CTL response [2]. The role of priming or tolerance induction by poly(I:C) and the role of type I IFN in bacterial infections is controversial. *Ifnar*^{-/-} mice subjected to CLP exhibited improved survival with use of antibiotics but worse survival without antibiotics, highlighting the role of type I IFN in controlling bacterial burden [4, 10]. This suggests an important role for type I IFNs in modulating anti-bacterial responses during CLP-induced polymicrobial sepsis. Further a distinct role for type I IFNs was observed in extracellular (group B streptococcus, *Staphylococcus pneumoniae*, *E. coli*) versus intracellular (*Listeria monocytogenes*) bacterial infection models, with IFN signaling being protective [12] and deleterious [13] respectively in these models. In light of the known role for poly(I:C) in affecting phagocytosis and induction of hetero-tolerance, in this study we questioned whether poly(I:C) priming would exhibit these characteristics in a model of polymicrobial sepsis, thereby affecting the progression and outcome of sepsis.

Materials and Methods

Animals

Wild type C57BL/6 mice were purchased from NCI and all mice were bred or housed at Michigan State University in rooms maintained at 22–24°C with 50% humidity and a 12-hr light-dark cycle. Mouse chow and water were provided *ad libitum* to all animals. All experiments were performed with age- and sex-matched mice (males between 8–12 weeks of age). Animal procedures were approved by Michigan state University Institutional Animal Care and Use Committee (IACUC) and conformed to NIH guidelines.

CLP surgery

Animals were subjected to CLP as described [14]. Briefly, mice were anaesthetized using intraperitoneal injection of xylazine (5 mg/kg) and ketamine (80 mg/kg). The cecum was exteriorized, ligated and punctured with a 16G needle. The cecum was then inserted back and peritoneal cavity sutured with 5.0 silk. Sham surgery wherein cecum was exteriorized but neither ligated nor punctured was used as control. All animals were given a

subcutaneous injection of 1 ml saline (pre-warmed to 37°C) after the surgery. For mortality studies, mice were observed for 7 days after surgery. In studies involving poly(I:C) priming, mice were injected with 50 µg HMW poly(I:C) (InvivoGen, tlr1-pic) or vehicle (PBS) 12 hours prior to surgery.

Sample Processing

At pre-determined time of harvesting, mice were euthanized using CO₂ asphyxiation. Peritoneal fluid, plasma and organs were harvested and processed as previously described [20]. Briefly, the peritoneal cavity was flushed with R10 media (RPMI 1640 with 10% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin and 55 µM β mercaptoethanol), the cells collected and processed for flow cytometry analysis. The first peritoneal wash was done in 4 ml media and supernatant saved for further analysis. The cavity was then lavaged twice with 10 ml media and cells from all washes collected and counted for further analysis. Blood was centrifuged at 300 ×g for 5 min and supernatant stored at -80°C for ELISAs. The organs were harvested, flash frozen and stored at -80°C. Spleen was crushed, subjected to RBC lysis and filtered through 40 µm nylon mesh. For further stimulations, cells were counted, resuspended in R10 at concentration of 10 × 10⁶ cells/ml and incubated at 37°C for 18 hours. For flow cytometric analysis, 2×10⁶ cells were used and processed.

Flow cytometry

Cells collected from experimental animals were stained with antibody cocktail made in 2.4G2 supernatant (FcγR blocking antibody) to block non-specific binding and washed with staining buffer (PBS with sodium azide and BCS). The antibodies against cell surface markers CD11b, F4/80, CD3, CD19, MHCII and Gr-1 were obtained from eBiosciences and used as per manufacturer's instructions. Cells were run on LSR II and data analyzed using Flowjo software [19]. Neutrophils were gated as CD3-CD19-CD11b+Gr-1+ cells, macrophages as CD3-CD19-CD11b+F4/80+ cells, B cells as CD19+ and T cells as CD19-CD3+ cells.

Cytokine/chemokine measurements

Cytokines were measured from plasma, splenic culture supernatant and peritoneal fluid using ELISA kits from eBiosciences, Inc. as per manufacturer's protocol [20].

Bacterial Counts

Bacterial load was determined in peritoneal fluid and blood [20]. Briefly, sample was serially diluted and plated on Mueller-Hinton Agar plates (Difco). The plates were then incubated at 37°C for 24 hours and the number of colony forming units (CFU) counted and recorded.

Quantitative RT-PCR

To determine the relative levels of a specific RNA transcript, RNA was isolated from snap frozen tissue using Qiagen RNeasy mini kit using manufacturers' protocol and as described earlier [20]. Reverse transcription was carried out with 1 µg of RNA using promega cDNA

synthesis kit. Q-RT-PCR was performed with ABI fast 7500 (Applied Biosystems) and all genes were normalized to HPRT.

Phagocytosis and ROS potential

Cells harvested from experimental mice, 12 hours post-surgery were used as source of neutrophils for phagocytic potential and ROS generation [15, 20]. Briefly, peritoneal cavity was lavaged with R2 media (RPMI 1640 with 2% FBS), and cells counted for the assay. For phagocytosis, pHrodo *E. coli* bioparticle conjugate (invitrogen) was used as described in manufacturer's manual. Briefly, 1×10^5 cells were incubated with bio-particles for 30 min at either 37°C (experimental) or 4°C (control) and reaction stopped by washing with cold FACS wash buffer. For ROS detection, 1×10^5 cells were preloaded with 5 mM DHR-123 dye (invitrogen) and stimulated with PMA (1 ng/ml). The cells were stained with Gr-1 antibodies to detect neutrophils and increase in MFI over control recorded as phagocytic potential and ROS generation, respectively.

Statistical Analysis

All experimental data in the figures is expressed as mean $\pm$ SEM and analyzed using GraphPad Prism Software. Each "N" represents individual mouse. Student's t-test (for comparing groups with equal variances) or Mann-Whitney (for comparing groups with unequal variances) was used to compare two experimental groups. Differences in the survival were determined using the log-rank test. P-values <0.05 were considered statistically significant.

Results

Poly(I:C) pretreatment enhances cecal ligation and puncture (CLP)-induced mortality

To assess the clinical and pathological consequence of poly(I:C) priming in polymicrobial sepsis, we followed the survival of mice in response to CLP surgery. Mice were injected with either PBS (vehicle control) or poly(I:C), 12 hours prior to surgery and monitored for survival for 7 days. As shown in Fig 1, while the PBS injected CLP group had a survival of ~80%, survival in the poly(I:C) treated group was significantly decreased (~40%). This data demonstrates that poly(I:C) priming and therefore TLR3 activation prior to CLP surgery enhances mortality in mice.

Systemic cytokine production

Increased pro-inflammatory response to a septic insult is correlative and indicative of early mortality [22]. To understand the mechanisms underlying the poor survival in poly(I:C) primed septic mice, we examined the effect of poly(I:C) priming on inflammatory cytokines in the plasma as well as in various organs.

Effect of poly(I:C) priming in sham mice—Poly(I:C) injection in the sham group led to significant increase in IL-10, IL-12p40 and MCP-1 compared to vehicle group. However, levels of IL-6, TNF α and IFN γ were not affected by poly(I:C) treatment in sham mice.

Effect of CLP in PBS injected groups—Plasma levels of IL-6, TNF α and MCP-1 were significantly enhanced in mice subjected to CLP compared to sham surgery in the PBS injected mice. (Fig 2). However, IL12-p40, and IL10 levels in the CLP and sham groups (PBS injected) at this time point were similar; and IFN γ was decreased in the CLP compared to sham.

Comparison of poly(I:C) and vehicle injected CLP mice—Poly(I:C) priming in CLP mice led to significantly higher plasma levels of TNF α , but not IL-6 when compared to PBS injected CLP mice. Additionally, plasma levels of IFN γ , IL-12p40 and MCP-1 were significantly enhanced in the poly(I:C) injected CLP group compared to PBS injected CLP mice. Plasma levels of IL-10 levels were similar between poly(I:C)- and PBS-injected CLP groups.

To further characterize the inflammatory response in different organs, we measured cytokine levels in lysates from lung, liver, spleen, kidney and heart tissues. Enhanced MCP-1 and IL-12p40 production was observed in poly(I:C) pre-treated CLP group in most organs (Table 1). Together, these data demonstrate that priming with poly(I:C) *in vivo* potentiates systemic inflammatory response subsequent to septic peritonitis.

Local cytokine production

To compare production of inflammatory cytokines at the site of infection, consequent to pretreatment with poly(I:C), we examined levels of IL-6, TNF α , IL12-p40, MCP-1, IL-10 and IFN γ in the peritoneal fluid from the four groups of mice. Similar to systemic response, local response too was altered by prior exposure to poly(I:C). Levels of TNF α , IL-12p40 and MCP-1 were significantly higher in septic mice primed with poly(I:C) compared to PBS pre-treated CLP mice. However, IL-6 and IL-10 levels were similar between the PBS- and poly(I:C) injected CLP group (Fig 3). Further, even though IL-12p40 was similar between PBS injected sham and CLP groups, poly(I:C) priming induced IL-12p40 production in response to septic insult. These data demonstrate that local cytokine production following CLP in the poly(I:C) pre-treated animals closely mirrors the trend observed in the plasma.

Bacterial Clearance

In addition to dysregulation of inflammatory cytokines, bacterial clearance is an important factor in mortality from sepsis. To examine if poly(I:C) priming affected bacterial clearance in CLP mice, we examined bacterial load in the peritoneal cavity of septic mice pretreated with either PBS or poly(I:C). As shown in Fig 4, poly I:C priming significantly increased bacterial burden in the peritoneal cavity suggesting a decrease in bacterial clearance potential. Note that there were no bacterial counts in the sham mice in either PBS or poly(I:C) injected groups (data not shown). To further assess if this was due to defects in phagocytic potential or in ROS generation, we collected peritoneal cells from the 4 groups of mice (PBS/poly(I:C) injected sham and CLP mice) and assessed the ability of these cells to phagocytose as well as examined the levels of ROS production by flow cytometry. As shown in 4b and 4c, poly(I:C) treatment did not affect either phagocytic potential or ROS generation in the CLP mice. However, in the sham group, poly(I:C) treatment enhanced phagocytic potential but slightly reduced ROS generation. Because, neither bactericidal

events were significantly altered between the septic groups this suggests that poly(I:C) priming reduced bacterial clearance without affecting phagocytosis and ROS generation.

Cellular infiltration and activation

Since the bactericidal functions of neutrophils were unaffected by poly(I:C) priming, we hypothesized that higher bacterial load could be due to lower cellular infiltration. To understand if poly(I:C) pretreatment differentially affects immune infiltration, we examined the numbers of neutrophils, and macrophages in the four groups of mice. In the PBS injected groups, CLP enhanced peritoneal neutrophil infiltration whereas it decreased peritoneal macrophage accumulation (Fig 5). Although poly(I:C) pretreatment did not affect neutrophil infiltration in either groups, macrophage numbers were markedly downregulated in sham following poly(I:C) treatment and importantly, this was observed in CLP group as well- i.e. poly(I:C) injected CLP mice had significantly lower numbers of macrophages compared to PBS injected CLP mice. Together, these results suggest that decreased peritoneal macrophages could be a reasoning behind higher bacterial load in this model.

To further look at the activation status of these cells, we assessed expression of CD11b (Mac-1) on neutrophils and macrophages and found that the expression of Mac-1 in sham mice was unaffected by poly(I:C); however, in the CLP mice, poly(I:C) treatment led to significantly reduced CD11b expression in macrophages (FIG 5b). Previous studies have shown that lack of CD11b is associated with poorer sepsis outcome with reduced bacterial clearance as well as higher systemic inflammation [11]. Thus, reduced CD11b combined with decreased macrophage numbers together could contribute to reduced bacterial clearance in poly I:C treated CLP group.

Discussion

Inflammatory status plays a key role in the pathogenesis of many inflammatory diseases and thus, it is not surprising that polymicrobial sepsis can be influenced by prior exposure to microbial ligands or live microbes. It is in this context that we demonstrate that priming mice with poly(I:C) prior to CLP surgery, leads to an exacerbated inflammatory response. However, it is clear from other studies that these effects are dependent on specific TLRs being activated for priming and the type of model that is being used. Priming with other TLR ligands, especially TLR2, TLR4 and TLR9 have been shown to improve immunopathology of sepsis. In a study using a CASP model of septic peritonitis, the authors showed that exposure to MALP2 (TLR2 ligand) several days prior to sepsis induction markedly increased the resistance to peritonitis. Protection in this case was associated with enhanced effector neutrophils and attenuated cytokine response, as well as upregulation of a negative regulatory receptor ST2 [7]. Similar to the pretreatment with TLR2 agonists, Shi et al [21] showed that LPS, a TLR4 ligand pretreatment alleviated multiple organ injury following CLP as well as improved survival in a rat model. This was accompanied by reduced NF κ B activation in the lungs and decreased systemic cytokine levels. Similar responses were noted by Wheeler et al [25] who also pretreated with LPS 18 hours prior to CLP and found that this improved bacterial clearance and improved mortality in mice. Pretreatment with CpG-ODN, a TLR9 agonist, has also been shown to improve survival

following CLP as well as decrease bacterial load both locally and systemically in mouse and rat models [17, 24]. Consistent with this, Gao et al [8] showed that CPG-ODN pretreatment (1 hour prior to CLP in mice) prevents CLP-induced cardiac dysfunction suggesting that modulating TLR9 could be an effective approach to treating cardiovascular dysfunction in sepsis. While these studies were done in adult animals, Wynn et al [26] showed that in neonates, TLR4 and TLR7/8 agonists also improved survival in the neonates following fecal slurry-induced sepsis.

Although some studies have looked at the role of TLR3 priming in host response to sepsis, these studies utilized varied approaches. Wynn et al [26] tested whether TLR3 agonist priming could improve host defense against sepsis in a neonatal model, where sepsis was induced by fecal slurry. Here pretreatment with TLR3 agonist (@10 µg/g body weight) for 24 hours prior to sepsis initiation had no effect on survival or bacteremia. Interestingly, however, similar to our study, survival was worsened with larger doses (30 µg/g body weight). In our studies, even though our doses were relatively much lower than that of Wynn et al, our mice succumbed more to CLP-induced sepsis, consequent to poly(I:C) priming. It should be noted that the sepsis model induced by Wynn et al, did not involve surgery that could attribute to the differences between the studies. However, consistent with ours, Wynn et al also observed robust inflammatory response albeit with shorter kinetics following poly(I:C) priming. Since the innate and adaptive systems of neonates are different compared to adults (used in our study), that could also account for the differences.

In a different study by Davis et al [3] Poly(I:C) treatment in mice subjected to CLP and exposed to secondary bacterial challenge, led to better survival. This however was route of administration specific. As noted by the authors, systemic administration of poly(I:C) did not affect survival [3]. This is in contrast to our study where we observe detrimental effect of poly(I:C) pre-treatment. We administered poly(I:C) 12 hours prior to CLP induction whereas Davis et al administered poly(I:C) 24 hours *post*-CLP. Moreover, the route of administration was also different (intranasal versus intraperitoneal in our study). It should also be noted that survival in CLP mice in the PBS group in our study was ~80% compared to only ~33% in the study by Davis *et al* and thus our model might be considered milder compared to that of Davis *et al*. Whether this difference can contribute to the differential effects is not known. Similar to the studies of Davis et al, Pyles et al [16] demonstrated that intranasal administration of poly(I:C) improved survival in a rabbit model of tularemia even though they were administered prior to infection. Together, these differences, underscore the importance of not generalizing the effect of poly(I:C) priming under septic conditions.

Other studies that have looked at primary single injuries in the context of TLR3 knockout or blockade in mice subjected to CLP [1]. In these studies, although TLR3 KO mice had higher inflammatory cytokines and neutrophil recruitment initially, the cytokines returned to baseline quickly in the KO mice, suggesting that TLR3 is important for sustained inflammation during septic peritonitis. In addition to the role of the receptor, IRF3 a signaling component of TLR3 was also shown to be a critical mediator of sepsis pathogenesis [23]. In line with these genetic tools for blocking TLR3 activity, administration of anti-TLR3 antibody also decreased sepsis-induced mortality [1]. Consistent with this, Gong et al [9] showed that a compound (FC-99) that suppresses TLR3 expression,

ameliorated CLP-induced sepsis in mice. This was associated with decreased inflammatory response as well as decreased bacterial load in the FC-99 treated mice.

While the mechanisms of poor survival in our model may be related to lower bacterial clearance and/or higher inflammatory response, what triggers either of these responses is a subject for future study. Further studies with comprehensive analysis of responses from different cell types is necessary to understand the mechanisms. Taken together, our studies demonstrate that poly(I:C) priming is detrimental to the host subjected to polymicrobial sepsis.

Acknowledgments

We gratefully acknowledge the support from NIH (grants HL095637, AI099404 and AR056680 to N.P.). We thank the University lab animal resources for taking excellent care of our animals, and the Histopathology laboratory for their excellent service.

References

1. Cavassani KA, Ishii M, Wen H, Schaller MA. TLR3 is an endogenous sensor of tissue necrosis during acute inflammatory events. *The Journal of*. 2008; 205(11):2609–2621. DOI: 10.1084/jem.20081370
2. Coffman RL, Sher A, Seder RA. Vaccine adjuvants: putting innate immunity to work. *Immunity*. 2010; 33(4):492–503. DOI: 10.1016/j.immuni.2010.10.002 [PubMed: 21029960]
3. Davis, Christopher G., Chang, Kathy, Osborne, Dale, Walton, Andrew H., Ghosh, Sarbani, Dunne, William Michael, Hotchkiss, Richard S., Muenzer, Jared T. TLR3 agonist improves survival to secondary pneumonia in a double injury model. *The Journal of surgical research*. 2013; 182(2):270–276. DOI: 10.1016/j.jss.2012.09.039 [PubMed: 23083640]
4. DeJager L, Vandevyver S, Ballegeer M, Van Wouterghem E, An LL, Riggs J, Kolbeck R, Libert C. Pharmacological inhibition of type I interferon signaling protects mice against lethal sepsis. *J Infect Dis*. 2014; 209(6):960–970. DOI: 10.1093/infdis/jit600 [PubMed: 24218508]
5. Deng T, Feng X, Liu P, Yan K, Chen Y, Han D. Toll-like receptor 3 activation differentially regulates phagocytosis of bacteria and apoptotic neutrophils by mouse peritoneal macrophages. *Immunol Cell Biol*. 2013; 91(1):52–59. DOI: 10.1038/icb.2012.45 [PubMed: 22986631]
6. Doyle SE, O'Connell RM, Miranda GA, Vaidya SA, Chow EK, Liu PT, Suzuki S, et al. Toll-like receptors induce a phagocytic gene program through p38. *J Exp Med*. 2004; 199(1):81–90. DOI: 10.1084/jem.20031237 [PubMed: 14699082]
7. Feterowski C. Attenuated pathogenesis of polymicrobial peritonitis in mice after TLR2 agonist pre-treatment involves ST2 up-regulation. *International Immunology*. 2005; 17(8):1035–1046. DOI: 10.1093/intimm/dxh282 [PubMed: 16000329]
8. Gao M, Ha T, Zhang X, Wang X, Liu L, Kalbfleisch J, Singh K, Williams D, Li C. The Toll-like Receptor 9 Ligand, CpG Oligodeoxynucleotide, Attenuates Cardiac Dysfunction in Polymicrobial Sepsis, Involving Activation of Both Phosphoinositide 3 Kinase/Akt and Extracellular-Signal-Related Kinase Signaling. *The Journal of infectious diseases*. 2013; 207(9):1471–1479. DOI: 10.1093/infdis/jit036 [PubMed: 23359590]
9. Gong, Wei, Hu, Erling, Dou, Huan, Song, Yuxian, Yang, Liu, Ji, Jianjian, Li, Erguang, Tan, Renxiang, Hou, Yayi. A novel 1,2-benzenediamine derivative FC-99 suppresses TLR3 expression and ameliorates disease symptoms in a mouse model of sepsis. *British Journal of Pharmacology*. 2014; 171(21):4866–4878. DOI: 10.1111/bph.12797 [PubMed: 24903157]
10. Kelly-Scumpia KM, Scumpia PO, Delano MJ, Weinstein JS, Cuenca AG, Wynn JL, Moldawer LL. Type I interferon signaling in hematopoietic cells is required for survival in mouse polymicrobial sepsis by regulating CXCL10. *J Exp Med*. 2010; 207(2):319–326. DOI: 10.1084/jem.20091959 [PubMed: 20071504]

11. Liu JR, Han X, Soriano SG, Yuki K. The role of macrophage 1 antigen in polymicrobial sepsis. *Shock*. 2014; 42(6):532–539. DOI: 10.1097/SHK.0000000000000250 [PubMed: 25075642]
12. Mancuso G, Midiri A, Biondo C, Beninati C, Zummo S, Galbo R, Tomasello F, et al. Type I IFN signaling is crucial for host resistance against different species of pathogenic bacteria. *J Immunol*. 2007; 178(5):3126–3133. [PubMed: 17312160]
13. O'Connell RM, Saha SK, Vaidya SA, Bruhn KW, Miranda GA, Zarnegar B, Perry AK, et al. Type I interferon production enhances susceptibility to *Listeria monocytogenes* infection. *J Exp Med*. 2004; 200(4):437–445. DOI: 10.1084/jem.20040712 [PubMed: 15302901]
14. Packiriswamy N, Lee T, Raghavendra PB, Durairaj H, Wang H, Parameswaran N. G-protein-coupled receptor kinase-5 mediates inflammation but does not regulate cellular infiltration or bacterial load in a polymicrobial sepsis model in mice. *J Innate Immun*. 2013; 5(4):401–413. DOI: 10.1159/000347002 [PubMed: 23485819]
15. Packiriswamy N, Steury M, McCabe IC, Fitzgerald SD, Parameswaran N. Bacterial Dose-Dependent Role of G Protein-Coupled Receptor Kinase 5 in *Escherichia coli*-Induced Pneumonia. *Infect Immun*. 2016; 84(5):1633–1641. DOI: 10.1128/IAI.00051-16 [PubMed: 26975990]
16. Pyles RB, Jezek GE, Eaves-Pyles TD. Toll-Like Receptor 3 Agonist Protection against Experimental *Francisella tularensis* Respiratory Tract Infection. *Infection and Immunity*. 2010; 78(4):1700–1710. DOI: 10.1128/IAI.00736-09 [PubMed: 20123717]
17. Rice, Lauren, Orlow, Daniel, Ceonzo, Katherine, Stahl, Gregory L., Tzianabos, Aurthur O., Wada, Hiromi, Aird, William C., Buras, Jon A. CpG oligodeoxynucleotide protection in polymicrobial sepsis is dependent on interleukin-17. *The Journal of infectious diseases*. 2005; 191(8):1368–1376. DOI: 10.1086/428452 [PubMed: 15776385]
18. Savva A, Roger T. Targeting toll-like receptors: promising therapeutic strategies for the management of sepsis-associated pathology and infectious diseases. *Front Immunol*. 2013; 4:387.doi: 10.3389/fimmu.2013.00387 [PubMed: 24302927]
19. Sharma D, Malik A, Steury MD, Lucas PC, Parameswaran N. Protective Role of beta-arrestin2 in Colitis Through Modulation of T-cell Activation. *Inflamm Bowel Dis*. 2015; 21(12):2766–2777. DOI: 10.1097/MIB.0000000000000563 [PubMed: 26296063]
20. Sharma D, Packiriswamy N, Malik A, Lucas PC, Parameswaran N. Nonhematopoietic beta-Arrestin-1 inhibits inflammation in a murine model of polymicrobial sepsis. *Am J Pathol*. 2014; 184(8):2297–2309. DOI: 10.1016/j.ajpath.2014.05.002 [PubMed: 24946011]
21. Shi, Dong-Wei, Zhang, Jing, Jiang, Hong-Ni, Tong, Chao-Yang, Gu, Guo-Rong, Ji, Yuan, Summah, Hanssa, Qu, Jie-Ming. LPS pretreatment ameliorates multiple organ injuries and improves survival in a murine model of polymicrobial sepsis. *Inflammation*. 2011; 60(9):841–849. DOI: 10.1007/s00011-011-0342-5
22. Stearns-Kurosawa DJ, Osuchowski MF, Valentine C, Kurosawa S, Remick DG. The pathogenesis of sepsis. *Annu Rev Pathol*. 2011; 6:19–48. DOI: 10.1146/annurev-pathol-011110-130327 [PubMed: 20887193]
23. Walker WE, Bozzi AT, Goldstein DR. IRF3 contributes to sepsis pathogenesis in the mouse cecal ligation and puncture model. *Journal of leukocyte biology*. 2012; 92(6):1261–1268. DOI: 10.1189/jlb.0312138 [PubMed: 23048204]
24. Weighardt H, Feterowski C, Veit M, Rump M, Wagner H, Holzmann B. Increased Resistance Against Acute Polymicrobial Sepsis in Mice Challenged with Immunostimulatory CpG Oligodeoxynucleotides Is Related to an Enhanced Innate Effector Cell Response. *Journal of immunology* (Baltimore, Md. : 1950). 2000; 165(8):4537–4543. DOI: 10.4049/jimmunol.165.8.4537
25. Wheeler, Derek S., Lahni, Patrick M., Denenberg, Alvin G., Poynter, Sue E., Wong, Hector R., Cook, James A., Zingarelli, Basilia. INDUCTION OF ENDOTOXIN TOLERANCE ENHANCES BACTERIAL CLEARANCE AND SURVIVAL IN MURINE POLYMICROBIAL SEPSIS. *Shock*. 2008; :1–7. DOI: 10.1097/shk.0b013e318162c190
26. Wynn, James L., Scumpia, Philip O., Winfield, Robert D., Delano, Matthew J., Kelly-Scumpia, Kindra, Barker, Tolga, Ungaro, Ricardo, Levy, Ofer, Moldawer, Lyle L. Defective innate immunity predisposes murine neonates to poor sepsis outcome but is reversed by TLR agonists. *Blood*. 2008; 112(5):1750–1758. DOI: 10.1182/blood-2008-01-130500 [PubMed: 18591384]

Fig 1. Pretreatment with Poly(I:C) enhances sepsis-induced mortality

Mice were injected with vehicle (PBS) or Poly(I:C) 12 hours prior to cecal ligation and puncture surgery. CLP was performed as described in the methods and survival of mice monitored for 7 days. * $P < 0.05$; $N = 11-13$

Fig 2. Pretreatment with Poly(I:C) enhances sepsis-induced systemic inflammation

Mice were injected with vehicle (PBS) or Poly(I:C) 12 hours prior to Sham/CLP surgery.

Mice were euthanized 6 hours after surgery and blood was collected as described in the methods. Plasma cytokine levels were measured using ELISA. N=13. *P<0.05; **P<0.01;

***P<0.001.

Fig 3. Poly(I:C) pretreatment enhances peritoneal cytokine levels following septic peritonitis Mice were injected with vehicle (PBS) or Poly(I:C) 12 hours prior to CLP surgery. Six hours after surgery, mice were euthanized and peritoneal lavage collected as described in the methods. Peritoneal lavage was collected and processed as described in the methods and cytokine levels were measured using ELISA. N=13. **P<0.01; ***P<0.001.

Fig 4. TLR3 priming dysregulates bacterial clearance in vivo

Peritoneal lavage from mouse groups described in Fig 3 were subjected to further analysis as shown. (a) Bacterial colonies were assessed as described in the methods. Note that the sham mice groups did not have any bacterial colonies. Phagocytic potential (b) and ROS generation (c) from neutrophils were assessed as described in the methods. N=13. *P<0.05; **P<0.01.

Fig 5. Poly(I:C) pretreatment reduces peritoneal macrophage numbers and activation following septic peritonitis

Peritoneal cells from lavage from the groups of mice described in Fig 3 were subjected to further flow cytometry analysis for determining the number and type of cells (A) and expression of CD11b in macrophages (MP) and neutrophils (PMNs). N=13. *P<0.05; ***P<0.01.

Table 1

Mice were injected with vehicle (PBS) or Poly(I:C) 12 hours prior to CLP surgery. Six hours after surgery, mice were euthanized and various organs collected and RNA extracted as described in the methods. Expression levels of various genes as indicated was determined by Q-RT-PCR. N=13.

	PBS SHAM	Poly(I:C) SHAM	PBS CLP	Poly(I:C) CLP
LUNG				
IL-12p40	1.6±0.3	2.53±0.4 *	1.03±0.07	2.3±0.2 ***
IL-10	25.7±5.2	25.4±5.1	25.42±2.2	21.6±2.6
TNFα			4.3±0.4	4.4±0.5
IL-6	3.0±0.4	2.7±0.4	16.45±2.9	16.5±3.6
IFNγ	1.4±0.2	1.4±0.2	0.9±0.1	0.9±0.1
MCP-1	10.87±2.9	15.4±3.4	24.33±1.7	62.8±8.7 ***
LIVER				
IL-12p40	1.5±0.02	2.6±0.2	1.0±0.2	2.5±0.4 ***
IL-10	35.45±2.6	36.4±1.8	38.6±7.7	49.6±15.6
TNFα	4.3±0.4	4.7±0.6	4.7±0.8	5.7±1.5
IL-6	4.0±0.2	4.0±0.2	15.95±1.8	15.8±1.6
IFNγ	2.2±0.2	2.3±0.1	0.8±0.03	1.0±0.06
MCP-1	22.07±1.6	65.8±3.0 ***	15.9±1.7	47.2±2.7 ***
SPLEEN				
IL-12p40	47.41±5.8	27.0±1.7	27.6±4.3	28.2±4.1
IL-10	87.5±46.8	80.8±14.1	73.3±19.3	155.6±42.6
TNFα			200.4±73.5	230.2±92.6
IL-6	31.5±10.1	37.4±4.8	49.4±13.4	73.2±23.4
MCP-1	87.5±46.8	80.8±14.1	3027±168.5	4429±69.1 ***
KIDNEY				
IL-12p40	2.28±0.2	3.9±0.1 **	1.7±0.1	4.4±0.3 ***
IL-10	39.5±3.9	39.2±3.5	36.3±4.5	31.3±3.6
TNFα	5.6±1.0	4.5±0.8	3.8±0.4	4.2±0.4
IL-6	5.3±0.5	5.7±0.6	21.2±0.8	21.7±1.0
IFNγ	2.7±0.2	2.4±0.2	1.4±0.1	1.3±0.1
MCP-1	22.1±2.7	32.5±2.0	28.8±3.1	53.6±2.5 ***
HEART				
IL-12p40	2.9±0.2	1.5±0.2	1.2±0.2	1.9±0.1 ***
IL-10	25±2.8	30.3±2.8	25.2±1.8	19.8±1.2

	PBS SHAM	Poly(I:C) SHAM	PBS CLP	Poly(I:C) CLP
TNFα	1.8 $\pm$ 0.5	2.6 $\pm$ 0.3	3.4 $\pm$ 0.3	3.2 $\pm$ 0.2
IL-6	3.2 $\pm$ 0.4	3.7 $\pm$ 0.2	9.6$\pm$1.3	9.8$\pm$1.4
IFNγ	1.7 $\pm$ 0.2	2.0 $\pm$ 0.04	1.2$\pm$0.1	1.0$\pm$0.05
MCP-1	12.1 $\pm$ 1.8	19.0 $\pm$ 0.9	35.3$\pm$2.1	37.6$\pm$2.0

*
P<0.05;

**
P<0.01;

P<0.001.