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Cutting Edge: Myeloid Differentiation Factor 88 Deficiency Improves Resistance Against Sepsis Caused by Polymicrobial Infection

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Toll-like receptors (TLRs) are important for the activation of innate immune cells upon encounter of microbial pathogens. The present study investigated the potential roles of TLR2, TLR4, and the signaling protein myeloid differentiation factor 88 (MyD88) in polymicrobial septic peritonitis. Whereas both TLR2 and TLR4 were dispensable for host defense against septic peritonitis, MyD88-deficient mice were protected in this infection model. Recruitment of neutrophils to the septic focus and bacterial clearance were normal in MyD88-deficient mice. In contrast, the systemic inflammatory response was strongly attenuated in the absence of MyD88. Surprisingly, MyD88 deficiency did not alter cytokine and chemokine production in spleen and liver, but markedly reduced the inflammatory response in peripheral organs. Production of monocyte chemotactic protein-1 and macrophage-inflammatory protein-1α was entirely independent of MyD88. These results imply a central role of MyD88 for the systemic immune pathology of polymicrobial sepsis and show that cytokine production in spleen and induction of certain chemokines are MyD88 independent. The Journal of Immunology, 2002, 169: 2823–2827.

Materials and Methods

Mouse strains and colon ascendens stent peritonitis (CASP) model of polymicrobial septic peritonitis

MyD88-deficient mice backcrossed eight times to the C57BL/6 background were kindly provided by Dr. S. Akira (Osaka, Japan) (13). TLR2-deficient mice were a kind gift of Tularik (South San Francisco, CA) (23). Control C57BL/6 mice, C3H/HeN (TLR4+/−) mice, and TLR4-deficient C3H/HeJ (TLR4−/−) mice were purchased from Harlan Winkelmann (Borchem, Germany). TLR2-deficient mice were backcrossed five times with the C3H/HeJ strain to obtain mice doubly deficient for TLR2 and TLR4 (TLR2−/−TLR4−/−) mice. At 8–12 wk of age were used for all experiments. The CASP procedure used for induction of septic peritonitis was described in detail previously (24).

Bacterial counts and peritoneal neutrophil accumulation

Mice were sacrificed before or 12 h after CASP and peritoneal lavage fluid was collected. Serial dilutions of lavage fluids were plated on blood agar plates. CFU were counted after incubation at 37°C for 24 h and calculated as CFU per whole peritoneal cavity. In addition, peritoneal lavage cells...
were counted and differentiated by staining with Abs against Mac-1 (M1/70) and Ly-6G/Gr-1 (RB6-8C5) using appropriate isotype-matched controls (all from BD PharMingen, San Diego, CA).

Analysis of cytokine and chemokine production
Peripheral blood, spleen, liver, and lung were collected before or 12 h after CASP. Peripheral organs were snap-frozen in liquid nitrogen and homogenized after thawing in 1 ml PBS containing complete protease inhibitors (Roche Diagnostics, Mannheim, Germany). Organ extracts were centrifuged (6000 × g for 20 min at 4°C) and mediator concentrations were measured in supernatants by ELISA specific for TNF, IL-10, IL-12, macrophage-inflammatory protein (MIP)-1α, MIP-2, cytokine-induced neutrophil chemoattractant (KC), or monocyte chemoattractant protein (MCP)-1 (all from R&D Systems, Minneapolis, MN). Immune mediator levels in peripheral organs were normalized against the protein concentration in each organ extract as determined by the bicinchoninic acid kit (Pierce, Rockford, IL).

Statistical analysis
Statistical analysis of the data was performed using the Mann-Whitney U test or the Student t test where appropriate. Survival data were analyzed by log-rank test. All data are presented as mean ± SEM. The level of significance was p < 0.05.

Results
MyD88-deficient mice are protected from the lethal effects of polymicrobial septic peritonitis
Mice deficient for TLR2, TLR4, or MyD88 are highly susceptible to monomicrobial infection with bacterial or viral pathogens (25–29). To elucidate the potential role of TLRs and MyD88 for the immune defense against polymicrobial infection, we analyzed gene-decient mice in a model of acute septic peritonitis. As demonstrated in Fig. 1 genetic deficiencies in TLR2 or TLR4 did not significantly alter survival of polymicrobial septic peritonitis. Survival of mice deficient for both TLR2 and TLR4 (TLR2<sup>−/−</sup>/TLR4<sup>−/−</sup>) also did not significantly differ from that of controls. In contrast, survival of MyD88-deficient mice was significantly improved when compared with wild-type controls (Fig. 1). The overall survival rate of MyD88 knockout mice was 64.3% as compared with 21.4% for wild-type mice.

Bacterial clearance and peritoneal neutrophil recruitment are not altered by MyD88 deficiency
To further explore host defense mechanisms in MyD88-deficient mice, bacterial counts were determined in the septic focus. As depicted in Fig. 2A, MyD88 knockout and control mice exhibited similar bacterial counts in peritoneal cavity (p = 0.224), indicating that bacterial clearance in polymicrobial septic peritonitis is not impaired by MyD88 deficiency.

The innate immune defense against septic peritonitis is critically dependent on effector neutrophils. Therefore, it was interesting to observe that the absolute numbers of neutrophils accumulating in the infected peritoneal cavity were comparable in MyD88-deficient and control mice (Fig. 2B). These results suggest that neutrophil recruitment to the primary site of infection is MyD88 independent.

Distinct effects of MyD88 deficiency on local and systemic cytokine production during polymicrobial septic peritonitis
High systemic levels of inflammatory cytokines may contribute to organ injury and shock during sepsis. To elucidate potential mechanisms of protection in MyD88-deficient mice, we investigated serum cytokine levels 12 h after induction of septic peritonitis. The results in Fig. 3A show that serum concentrations of TNF, IL-12, and IL-10 were significantly increased in septic as compared with nonseptic control mice but remained low in septic MyD88-deficient mice. Production of IL-10 in response to septic peritonitis was also substantially impaired in MyD88 knockout mice, although a significant release of small amounts was detected (Fig. 3A). Interestingly, the systemic inflammatory response as measured by serum KC levels was not altered in mice deficient for either TLR2 or TLR4 but was weakly reduced in TLR2/TLR4 double-deficient mice. In contrast, systemic KC levels were close to baseline in MyD88-deficient mice (Fig. 3B).

Next, we examined the role of MyD88 for the cytokine response in different anatomic compartments by determining the concentrations of TNF, IL-12, and IL-10 in whole-organ protein extracts (Fig. 4). In septic control mice, TNF and IL-10 levels were elevated at comparable levels both in liver, lung, and spleen, whereas significant up-regulation of IL-12 was observed only in liver.
However, in MyD88 knockout mice the regulation of cytokine production showed marked differences in the organ compartments tested. Whereas the sepsis-induced increase of TNF and IL-10 levels in liver and lung was almost completely abrogated by MyD88 deficiency, production of these cytokines in spleen appeared not to be affected in MyD88-deficient mice (Fig. 4). These results suggest a distinct regulation of cytokine production in spleen.

Chemokines play a pivotal role in the innate immune response to experimental sepsis. Therefore, we measured chemokine production in protein extracts of liver, lung, and spleen. The results in Fig. 5 demonstrate that, in all organs tested, sepsis-induced production of the CC chemokines MCP-1 and MIP-1α did not significantly differ between MyD88 knockout and control mice, implying that the induction of these chemokines is largely independent of MyD88. In contrast, the CXC chemokines KC and MIP-2 were significantly reduced in liver and lung of MyD88-deficient mice as compared with wild-type mice. Consistent with the results of cytokine analysis (Fig. 4), production of KC and MIP-2 was also not significantly altered in spleens of septic MyD88-deficient mice (Fig. 5).

Discussion

The present report reveals new insights into the role of MyD88 for the complex immune pathology of polymicrobial sepsis. We demonstrate that MyD88 deficiency renders mice more resistant to the lethal effects of sepsis after polymicrobial peritonitis. Although previous reports have shown that MyD88-deficient mice are highly susceptible to monomicrobial infection with Staphylococcus aureus (26) or Toxoplasma gondii (30), the infection models applied may provide an explanation for these different observations. MyD88-dependent TLR signaling appears to be required for activation of protective immune responses during infection with low amounts of a single bacterial pathogen. In contrast, acute polymicrobial sepsis may expose innate immune cells to large amounts of diverse microbial components. Under these conditions, MyD88-dependent signaling may exceed the levels required for efficient host defense and may instead contribute to the detrimental hyperinflammation. However, depending on the type and dosage of pathogen applied, it is conceivable that MyD88-deficient mice may also exhibit increased resistance to certain models of monomicrobial sepsis. Therefore, prevention of an uncontrolled inflammatory response may contribute, at least in part, to the protective effects of...
MyD88 deficiency in polymicrobial sepsis. Our results showing that mice deficient for MyD88, but not TLR2 and/or TLR4, exhibit markedly reduced systemic cytokine levels during polymicrobial sepsis support this view.

MyD88-deficient mice have been reported to resist hyperinflammation and lethal shock after administration of high-dose LPS (14). The sepsis model applied in this study clearly differs from such toxic shock models by challenging mice with a large number of diverse bacterial pathogens. As a consequence, beneficial effects for the outcome of sepsis caused by polymicrobial infection may not only require attenuation of the systemic hyperinflammatory response but may also depend on the activation of efficient anti-bacterial defense mechanisms. Our results showing efficient bacterial clearance in MyD88 knockout mice are consistent with this notion. It appears likely that the intact bacterial clearance in septic MyD88-deficient mice may be explained, at least in part, by their unaltered peritoneal neutrophil accumulation.

The results of the present report also reveal new information about the role of MyD88 for the production of inflammatory mediators during infectious processes in vivo. We provide evidence for the existence of MyD88-dependent as well as MyD88-independent pathways of cytokine and chemokine production during polymicrobial septic peritonitis. The sepsis-induced up-regulation of cytokines and chemokines was found to be normal in spleen of MyD88 knockout mice but was almost completely abolished in lung and liver. These results indicate that the requirement of MyD88 for the local production of immune mediators during polymicrobial sepsis is dependent on the anatomical compartment involved. Recent work has identified a splice variant of MyD88 that acts as a dominant negative inhibitor of NF-κB activation by IL-1 and LPS and is the predominant form of MyD88 in spleen but not in other organs (31). Together, these observations suggest that mainly MyD88-independent pathway(s) may mediate cytokine production in spleen.

In addition, we have observed that both wild-type and MyD88-deficient mice challenged by septic peritonitis exhibited a comparable production of the CC chemokines MCP-1 and MIP-1α in all organs tested, whereas production of CXC chemokines (MIP-2, KC) and cytokines (TNF, IL-12, IL-10) was strongly impaired in liver and lung, but not spleen, of MyD88-deficient mice. Thus, these results further suggest that, during polymicrobial sepsis, distinct subsets of immune mediators can be defined that are produced either in a MyD88-independent or a MyD88-dependent manner. These results are also consistent with previous findings indicating that production of inflammatory proteins including IFN-β, IFN-γ-inducible protein-10, MCP-5, and inducible NO synthase by macrophages in vitro does not require MyD88 (21, 22).

The present study investigated not only the role of the common signaling adapter protein MyD88 but also the role of TLR2 and TLR4 in polymicrobial sepsis. We found that TLR2 and TLR4, either as individual receptors or in conjunction, are dispensable for the host defense against septic peritonitis. Moreover, combined deficiency of both TLR2 and TLR4 had only minor effects on the systemic inflammatory response as measured by serum KC levels. These findings suggest that other microbial agonists such as flagellin or bacterial DNA may also play a pathogenic role. Numerous TLRs may be triggered during polymicrobial sepsis, thereby rendering individual TLRs dispensable for immune activation and stimulation of host defense mechanisms. In addition, innate immune receptors other than TLRs may be activated.

In summary, the role of TLRs for host defense during infection was studied in a model of polymicrobial sepsis. We show that the genetic deficiency of MyD88, but not of TLR2 and TLR4, results in a significant survival benefit. Importantly, the local production of immune mediators in peripheral organs was found to be partially MyD88 independent. The molecular characterization of these MyD88-independent pathways may provide new insights into the regulation of the innate immune response to severe infection.

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References


