Prostaglandin E$_2$ Protects Against Liver Injury After *Escherichia coli* Infection but Hampers the Resolution of the Infection in Mice

Manabu Takano, Hitoshi Nishimura, Yuki Kimura, Junji Washizu, Yasujii Mokuno, Yuji Nimura and Yasunobu Yoshikai

*J Immunol* 1998; 161:3019-3025; 
http://www.jimmunol.org/content/161/6/3019

---

**References**

This article cites 56 articles, 28 of which you can access for free at: http://www.jimmunol.org/content/161/6/3019.full#ref-list-1

---

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

---

**Permissions**

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

---

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Prostaglandin \( \text{E}_2 \) Protects Against Liver Injury After *Escherichia coli* Infection but Hampers the Resolution of the Infection in Mice

Manabu Takano,* Hitoshi Nishimura,* Yuki Kimura,* Junji Washizu,* Yasujii Mokuno,* Yuji Nimura,† and Yasunobu Yoshikai†*

cyclic AMP-increasing agents such as prostaglandin \( \text{E}_2 \) (PGE\(_2\)) are known to protect against LPS-induced liver injury by down-regulating the production of inflammatory cytokines such as TNF-\( \alpha \). However, the effects of such reagents on host defense against bacterial infection remain unknown. We show here that in vivo administration of PGE\(_2\) significantly protected mice against liver injury after *Escherichia coli* infection but hampered the resolution of the infection. PGE\(_2\) significantly suppressed circulating TNF-\( \alpha \) and IL-12 levels but increased the IL-10 production after *E. coli* challenge. PGE\(_2\) inhibited the emergence of \( \gamma \delta \) T cells in the peritoneal cavity, which are important for host defense against *E. coli*, and deteriorated bacterial exclusion in the peritoneal cavity after *E. coli* challenge. These results suggested that PGE\(_2\) affects host defense mechanisms against *E. coli* infection through modulation of cytokine production and \( \gamma \delta \) T cell accumulation.

Cytokines are an important component of host defense against bacterial infection. Inflammatory cytokines such as IL-1, IL-6, IL-12, and TNF-\( \alpha \) are secreted by phagocytes in response to infection (1–3). The combined local effects of these cytokines result in an inflammatory response, which is usually one of the immediate local reactions to infection. IL-12 in synergy with TNF-\( \alpha \) can elicit production of large amounts of IFN-\( \gamma \) by NK cells and \( \gamma \delta \) T cells; this secreted IFN-\( \gamma \) is crucial in controlling infections before CD4 Th1 cells can be activated to produce cytokine (3). However, excessive inflammation responses sometimes induce tissue damage. Indeed, massive TNF-\( \alpha \) release from macrophages plays a central role in endotoxin-induced liver injury (4–6). TNF-\( \alpha \) causes intravascular coagulation and thereby ischemia of the organs, resulting in massive necrosis of the liver (6). TNF-\( \alpha \) is also known to induce apoptosis of hepatocytes directly (7). IFN-\( \gamma \) produced by NK cells and T cells potentiates the TNF-\( \alpha \) induced apoptosis in liver (8). Hence, it has been shown that IL-12 plays important roles in LPS-induced liver injury via stimulation of IFN-\( \gamma \)-producing cells (9).

A number of regulatory cytokines such as IL-4, IL-10, and TGF-\( \beta \) are described as having the capacity to block macrophage functions, including TNF-\( \alpha \) release (10–12). These cytokines are termed “macrophage-deactivating cytokines.” Exogenous IL-10 protects mice from septic shock and LPS-induced liver injury; conversely, anti-IL-10 mAb treated mice exhibit an increased susceptibility to LPS, suggesting that IL-10 plays a protective role in endotoxic shock though the inhibition of excessive TNF-\( \alpha \) production (13–15). Thus, excessive inflammatory responses are regulated by the macrophage-deactivating cytokines such as IL-10.

Several cytokines involved in LPS-induced liver injury, such as TNF-\( \alpha \), IL-10, IL-12, IFN-\( \gamma \), and IL-1\( \beta \), are reported to be regulated by a cAMP-dependent signaling pathway (16–21). Elevated levels of cAMP are known to inhibit activation of nuclear factor (NF-\( \kappa \)B) via retarded degradation of inhibitory factor- \( \kappa \)B (IF-\( \kappa \)B\(_{\text{B}}\)), while they stimulate activating transcription factor/cAMP response element (ATF/CRE) site-mediated gene transcription (22–25). Production of TNF-\( \alpha \) and IL-12 is inhibited by cAMP elevating agents, since their genes contain an NF-\( \kappa \)B site in the 5’ regulatory region, whereas IL-10 is up-regulated by the camp-dependent pathway, since the IL-10 gene contains a CRE/ATF-1-like site but no NF-\( \kappa \)B site in the 5’ regulatory region (19, 24, 26). PGE\(_2\), which activates membrane adenylate cyclase, has been demonstrated to elevate intracellular cAMP and consequently to activate cAMP-dependent protein kinase, resulting in down-regulation of LPS-induced TNF-\( \alpha \) production at the transcriptional and/or translational level (27–31). It has been demonstrated that PGE\(_2\) increases LPS-induced IL-10 production by peritoneal macrophages (28) and inhibits IL-12 production in an IL-10-independent manner (26). These findings suggest that PGE\(_2\) can be used as an anti-inflammatory agent in patients with fulminant and subfulminant viral hepatitis (32–37). However, inflammatory cytokines are important for control of infection with various pathogens. Therefore, it would be of interest to know whether PGE\(_2\) affects the host defense against microbial infection.

In the present study, we focused on the effect of PGE\(_2\) on host defense against infection with *Escherichia coli*, a Gram-negative bacterium, the cell wall components of which contain LPS. Our results demonstrated that PGE\(_2\) significantly suppressed circulating TNF-\( \alpha \) and IL-12 and reversely increased IL-10, consequently

---

*Laboratory of Host Defense and Germfree Life, Research Institute for Disease Mechanism and Control, Nagoya University School of Medicine, Nagoya, Japan

†Address correspondence and reprint requests to Dr. Yasunobu Yoshikai, Laboratory of Host Defense and Germfree Life, Research Institute for Disease Mechanism and Control, Nagoya University School of Medicine, 65 Tsurumai-cho Showa-ku Nagoya 466, Japan. E-mail address yyyoshika@tsuru.med.nagoya-u.ac.jp

Received for publication December 22, 1997. Accepted for publication May 11, 1998.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked according to 18 U.S.C. Section 1734 solely to indicate this fact.

† This work was supported in part by grants (to Y. Y.) from the Ministry of Education, Science, and Culture and the Ministry of Health and Welfare of Japan.

‡ Address correspondence and reprint requests to Dr. Yasunobu Yoshikai, Laboratory of Host Defense and Germfree Life, Research Institute for Disease Mechanism and Control, Nagoya University School of Medicine, 65 Tsurumai-cho Showa-ku Nagoya 466, Japan. E-mail address yyyoshika@tsuru.med.nagoya-u.ac.jp

Copyright © 1998 by The American Association of Immunologists

0022-1767/98/$02.00

---

3 Abbreviations used in this paper: ATF, activating transcription factor; CRE, cAMP response element; ALT, alanine aminotransaminase; β-CD, β-cyclohextrin; dbcAMP, dibutyryl cAMP; PGE\(_2\), prostaglandin \( \text{E}_2 \); \( \gamma \delta \), \( \gamma \delta \) T cell; PE, phycoerythrin; PEC, peritoneal exudate cells.
protecting against liver injury following *E. coli* infection. However, PGE₂ deteriorated the host defense mechanism against *E. coli* infection accompanied by inhibition of the emergence of γδ T cells. The implications of these findings for the mechanisms whereby PGE₂ affects host defense against *E. coli* infection were discussed.

### Materials and Methods

#### Animals and microorganisms

C3H/HeN mice were purchased from Japan SLC (Shizuoka, Japan). These mice were bred in our institute under specific pathogen-free conditions. Eight- to ten-week-old female mice were used for the experiments. *E. coli* (American Type Culture Collection No. 26; Manassas, VA) grown in brain-heart infusion broth (Difco Laboratories, Detroit, MI), was washed repeatedly, resuspended in PBS, and stored at −80°C in small aliquots until use. The concentration of bacteria was quantitated by plate counts.

#### Abs and reagents

Biotin-conjugated anti-CD3ε mAb, FITC-conjugated anti-TCRαβ mAb, and phycoerythrin (PE)-conjugated anti-TCRγδ mAb were purchased from PharMingen (San Diego, CA). Red-613-conjugated streptavidin was purchased from Life Technologies (Gaithersburg, MD). Prostaglandin E₁, β-cyclodextrin (PGE₂), and β-cyclodextrin (β-CD) were provided by Ono Chemical (Osaka, Japan). PGE₂ includes 7.69% prostaglandin E₂, mAbs against murine anti-TCRγδ mAb (UC7-13D5) were kindly provided by Dr. J. A. Bluestone (University of Chicago, Chicago, IL). Isotype control Ab was hamster anti-2,4,6-trinitrophenyl mAb, which were obtained by growing hybridoma cells in serum-free medium (medium 101, Nissui Pharmaceutical, Tokyo, Japan) and collecting the supernatant. Abs were then centrifuged and purified by 50% ammonium sulfate precipitation. The purity of the preparation was confirmed by SDS-PAGE, and the concentration of Ab was determined by the Lowry method. The mAbs, diluted to 1 mg/ml in PBS, were stored at −70°C until used. Two hundred micrograms of mAbs in 500 μl was injected into the peritoneal cavity on day 3 before primary infection with *E. coli*. In the control group, 200 μg of control Ab was injected.

#### Treatment of mice

Mice were injected with PGE₂ dissolved in ethanol (2.6 mg/mouse in 500 μl) or with the same dose of β-CD (as control) dissolved in ethanol diluted in PBS (final ethanol concentration was 9%). Three hours after PGE₂ or β-CD challenge, mice were inoculated with *E. coli* at a dose of 1.0 × 10⁸ CFU/mouse (1/5 LD₅₀) in 1.0 ml of PBS. After being assessed for serum transaminase activity, mice were inoculated with *E. coli* at a dose of 1.0 × 10⁹ CFU/mouse (1/5 LD₅₀) in 1.0 ml of PBS. All injections were performed i.p. and incubated fo r1 h at 37°C with RPMI 1640 medium containing 10% FCS. The sorted T cells (3 × 10⁵/well) were incubated in the anti-TCRγδ mAb-coated plates in the presence of murine rIL-12 (Genzyme). During the last 8 h of incubation, 1.0 μCi of [³H]thymidine incorporation was determined by scintillation counting. IFN-γ and IL-4 levels in the culture supernatants were determined by ELISA (Genzyme).

#### Statistical analysis

Data were analyzed by Student’s *t* test, and a Bonferroni correction was applied for multiple comparison. The value of *p* < 0.05 was considered statistically significant.

### Results

**PGE₂ protects against liver injury after *E. coli* infection**

cAMP-increasing agents are known to protect against LPS-induced liver injury (15, 34–37). We first examined whether administration of PGE₂ protects liver against liver injury following infection with a high dose of *E. coli*. A lethal dose of *E. coli* (1 × 10⁹ CFU/mouse) was injected i.p. into mice 3 h after an i.p. injection of 130 mg/kg of PGE₂ or β-CD. As shown in Figure 1, liver injury was induced after *E. coli* infection, as assessed by serum ATL activities. PGE₂ treatment provided significant protection against *E. coli*-induced liver injury compared with control β-CD (*p* < 0.05). The 130 mg/kg of PGE₂ was an optimal dose for protection against the liver injury; the maximal effect was obtained when PGE₂ was given 3 h before *E. coli* challenge.

Histologic examination also showed that challenge with a lethal dose of *E. coli* caused marked infiltration of inflammatory cells in the liver and massive necrosis of hepatocytes, characterized by cell swelling and focal change, showing cell shrinkage, chromatin condensation, and hemorrhage. Pretreatment with PGE₂ reduced these histologic changes (Fig. 2).
Effect of PGE$_2$ on the bacterial growth in the organ after inoculation with $E$. coli

We next examined bacterial growth to determine whether or not in vivo administration of PGE$_2$ was protective against infection with $E$. coli. A $1/5 \times LD_{50}$ dose ($1 \times 10^8$ CFU/mouse) of $E$. coli was inoculated i.p. in mice 3 h after an i.p. injection of 130 mg/kg of PGE$_2$ or the same amount of $\beta$-CD, and bacterial growth in the peritoneal cavity, liver, and spleen were examined 3 days later. As shown in Figure 3, the PGE$_2$ treatment exaggerated the bacteria growth following $E$. coli challenge. This result suggests that exogenous PGE$_2$ reduces host defense against $E$. coli infection.

Effects of PGE$_2$ on the emergence of $\gamma \delta$ T cells in the peritoneal cavity after $E$. coli infection

A prominent increase in $\gamma \delta$ T cells was observed in the peritoneal cavity after an i.p. infection with $E$. coli in C3H/He mice (38). We examined the effect of PGE$_2$ on the influx of $\gamma \delta$ T cells in the peritoneal cavity after $E$. coli inoculation. There was no difference in the number of $\gamma \delta$ T cells in the peritoneal cavity between control and PGE$_2$-treated mice.

Effects of PGE$_2$ on the serum TNF-$\alpha$, IL-12, and IL-10 level after $E$. coli challenge

PGE$_2$ is reported to inhibit TNF-$\alpha$ and IL-12 production but enhance IL-10 synthesis by macrophages in response to LPS stimulation (16, 26, 28). Therefore, the in vivo effect of PGE$_2$ on cytokine production was examined in the serum of mice after $E$. coli infection. As shown in Figure 4, serum TNF-$\alpha$ and IL-12 levels were maximal in control mice 3 h after $E$. coli infection, while these levels were significantly suppressed by in vivo administration of PGE$_2$ ($p < 0.05$). On the other hand, levels of IL-10 were increased only marginally in control mice after $E$. coli infection, but PGE$_2$ treatment significantly enhanced IL-10 release after $E$. coli challenge.
in the numbers of polymorphonuclear cells, lymphocytes, and macrophages in the peritoneal cavity on day 3 after *E. coli* infection between mice pretreated with or without PGE₂ (data not shown). Flow cytometry analyses of the expression of TCRαβ, TCRγδ, and CD3ε were conducted on the plastic-nonadherent PEC on day 3 after inoculation (Fig. 5A). A representative result from five mice for expression of TCRαβ and γδ is shown in Figure 5A, after gating of CD3⁺ T cells. Consistent with previous findings (34), the percentage of γδ T cells increased markedly to 82.0 ± 3.5% in CD3⁺ T cells from <5% before *E. coli* challenge in control mice, whereas it increased only to 39.2 ± 2.9% in PGE₂-treated mice. Absolute numbers of γδ T cells in the peritoneal cavity were significantly less in PGE₂-treated mice than in control mice (Fig. 5B, p < 0.05). Thus, PGE₂ severely inhibits the emergence of γδ T cells in the peritoneal cavity after *E. coli* infection. Although a dominant γδ T cell response to *E. coli* infection was observed, the protective role of the γδ T cells in *E. coli* infection remains to be elucidated. To this end, we first examined cytokine production by γδ T cells in the presence of IL-12, which is known to induce IFN-γ production by both resting and activated NK and T cells (3). As shown in Figure 6, IFN-γ production was only marginal when the γδ T cells were cultured on the anti-TCR γδ mAb-coated plates without IL-12, while IL-12 induced consider-

able IFN-γ production by the γδ T cells. IL-4 production was not detected in the culture of the γδ T cells. To obtain direct evidence for a protective role of γδ T cells in *E. coli* infection, we examined bacterial resolution in mice depleted of γδ T cells by treatment with anti-TCRγδ mAb. γδ T cells were confirmed to be almost completely depleted in the peritoneal cavity 3 days after infection with 1 × 10⁸ *E. coli* bacteria in mice pretreated with 200 μg of anti-TCRγδ mAb. (Fig. 7A). As shown in Figure 7B, a significant increase in the number of *E. coli* bacteria was evident in the peritoneal cavity and liver of anti-TCRγδ mAb-treated mice compared with control mAb-treated mice. These results indicate that the γδ T cells are important for protection against *E. coli* infection.

**Discussion**

PGE₂ and other cAMP-increasing agents such as dibutylryl cAMP (dbcAMP) are known to modulate the production of several cytokines such as TNF-α, IL-12, IL-1β, and IL-10 (16–21). Our results show that administration of PGE₂ decreased TNF-α and IL-12 production but enhanced IL-10 release in the serum following *E. coli* challenge. An NF-κB site is missing in the 5′ regulatory region of the IL-10 gene, whereas TNF-α and IL-12 genes contain such a site (19, 24, 26). PGE₂ may directly down-modulate TNF-α and IL-12 production by inhibition of NF-κB activation and up-regulate IL-10 production through stimulation of CRE/ATF-mediated gene transcription. IL-12 is known to be a potent stimulator
of IFN-γ production by NK and T cells (3), whereas IL-10 shuts down IFN-γ synthesis by these cells (10). Thus, it appears that aberrant cytokine production by PGE2 administration affects the mutual regulation of TNF-α, IL-12, and IL-10 in their synthesis after E. coli infection.

A protective effect of TNF-α administration or a lethal effect of depletion of TNF-α or TNFR by Ab treatment or gene mutation has been observed in mice infected with Listeria monocytogenes, Salmonella, or endogenous bacteria (2, 39–42). Thus, TNF-α is produced by macrophages in response to several Gram-positive and -negative bacteria and is important for protection against bacterial infection. IL-12 is also produced by macrophages stimulated with such bacteria and exerts various biologic effects, including enhanced proliferation and cytolytic activity of NK and Th1 cells as well as induction of IFN-γ, which are important for host defense against the infection (3). On the other hand, IL-10, which is produced by macrophages and T cells, is a potent inhibitor of macrophage-derived inflammatory cytokine synthesis (10). Since the balance of these cytokines released by macrophages is important to the induction of host defense, modulation of TNF-α, IL-12, and IL-10 release from infected macrophages is mainly responsible for the impaired host defense against E. coli infection induced by PGE2 administration.

TNF-α plays a pivotal role not only in host defense but also in the pathogenesis of liver failure, which is caused by endotoxin/LPS derived from Gram-negative bacteria (2, 6). IL-12 is also reported to be involved in LPS-induced liver injury, presumably by activation of NK and T cells capable of IFN-γ production and Fas-mediated cytotoxicity (9). PGE2 severely suppressed the level of circulating IL-12, in addition to TNF-α, following E. coli challenge. The suppression of both IL-12 and TNF-α may be a mechanism whereby PGE2 inhibits liver injury. Exogenous IL-10 effectively protects mice from lethal endotoxemia (13, 14). There is evidence for the involvement of IL-10 in the differential deactivation of murine peritoneal macrophages by PGE2 (43, 44). We have also reported that a cAMP-increasing agent, dbcAMP, not only decreases circulating TNF-α but also increases circulating IL-10 after LPS challenge in Propionibacterium acnes-treated mice and consequently protect these mice from LPS-induced liver injury.

FIGURE 6. IFN-γ production by γδ T cells upon TCR stimulation in the presence of IL-12. Purified γδ T cells (5 × 10^4/well) from mice infected with E. coli 3 days previously were incubated in anti-TCRγδ mAb-coated 96-well plates with indicated doses of IL-12 for 24 h at 37°C and the culture supernatants collected. The cytokine activity in the culture supernatant was determined for the presence of IFN-γ or IL-4 by ELISA. The data are representative of two separate experiments and are expressed as the means of triplicates ± SD. IL-4 was not detected in any culture supernatant. Significantly different from the value for control mice: *p < 0.05; **p < 0.01.

FIGURE 7. Effect of in vivo depletion of γδ T cells on recovery of bacteria from the peritoneal cavity and spleen after E. coli infection. A, C3H/He mice were inoculated i.p. with 200 μg of anti-TCRγδ mAb on day −3, and 2 × 10^8 E. coli on day 0. Plastic-nonadherent PEC on day 3 after an i.p. E. coli infection were stained with anti-TCRαβ, TCRγδ, and CD3ε mAb, and expression of TCRαβ vs TCRγδ was shown after gating of CD3ε cells. B, The number of E. coli recovered from the peritoneal cavity and liver of infected mice on day 3 was determined by colony formation assay on tryptic soy agar. Values are the mean ± SD for five mice of each group. Significant difference from the value for control mice: *p < 0.05.
modium falciparum, and herpes simplex virus (51–54). Similarly, this manuscript. We also thank Mr. Y. Yamakawa for technical assistance

doing. Using mice depleted of gd anti-TCR

determined. Using mice depleted of gd anti-TCR

prominent increases in gd-infected mice. An increase in intracellular cAMP levels up-
gd-infected mice against lipopolysaccharide-in-

bacterial growth after infection.

Dominant TCRαβ T cell response to infections with various microbial pathogens suggests that a significant fraction of gd T cells represents a first line of host defense against infections with diverse pathogens in nature (45–51). Takada et al. have reported prominent increases in gd T cells in the peritoneal cavity of particular strains including C3H/He after E. coli infection (38). However, the roles of the gd T cells in E. coli infection remain to be determined. Using mice depleted of gd T cells by treatment with anti-TCRαβ mAb or by C5 gene targeting, gd T cells are shown to contribute to host defense against infection with some parasites including Mycobacterium tuberculosis, L. monocytogenes, Plasmodium falciparum, and herpes simplex virus (51–54). Similarly, with mice depleted of gd T cells, we show here a protective role for gd T cells in E. coli infection. Furthermore, the emergence of gd T cells after E. coli infection was suppressed, albeit partially, by PGE2, administration, and bacterial growth was exaggerated in these mice. IL-12 is a major inducer of differentiation of Th1 cells producing IFN-γ, IL-2, and TNF-β, while suppressing the development of Th2 cells secreting IL-4 and IL-5 (3). Our results reveal that the gd T cells appearing during E. coli infection were mainly Th1-type cells, which were stimulated to produce IFN-γ in the presence of IL-12 (47, 50). IL-12 was shown to activate gd T cells to produce IFN-γ in synergy with TNF-α (55). On the other hand, IL-10 inhibits Th1 development by shutting down IFN-γ synthesis (10). Therefore, we speculate that suppression of IL-12 and TNF-α is responsible for the impaired accumulation of gd T cells in E. coli-infected mice. An increase in intracellular cAMP levels up-regulates type 2 cytokines, and Th2-type cell lines maintain higher levels of cAMP per cell than do Th1-type cell lines (18, 56–58). PGE2 not only affects the balance of TNF-α and IL-10 in macrophages, but also affects Th1-type cytokines such as IFN-γ and Th2-type cytokines such as IL-4 (56, 57, 58). Therefore, it is also possible that gd T cells appearing in the peritoneal cavity after E. coli infection are skewed to Th2-type cells producing IL-4 and that the Th1 response of the gd T cells is suppressed. Additional experiments are required to clarify this possibility.

In conclusion, PGE2 affects not only liver injury but also the host defense mechanism during E. coli infection through modulating inflammatory and anti-inflammatory cytokine production.

Acknowledgments

We thank Ono Chemical and Dr. J. A. Bluestone for providing PGE2, and UC7-13D5 hybridoma, respectively, and Dr. Daniel Murozek for reading this manuscript. We also thank Mr. Y. Yamakawa for technical assistance with the EPICS sorting.

References


3. Trinchieri, G. 1995. Interleukin-12: a proinflammatory cytokine with immuno-


