IFN-\(\gamma\) is a master regulator of endotoxin shock syndrome in mice primed with heat-killed Propionibacterium acnes

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Abstract

Hyper-coagulation, hypothermia, systemic inflammatory responses and shock are major clinical manifestations of endotoxin shock syndrome in human. As previously reported, mice primed with heat-killed Propionibacterium acnes are highly susceptible to the action of LPS to induce tumour necrosis factor (TNF-\(\alpha\)) and to that of TNF-\(\alpha\) to trigger lethal shock. Here we investigated the mechanisms underlying the \(P.\) acnes-induced sensitization to LPS and TNF-\(\alpha\) and the development of individual symptoms after subsequent challenge with LPS or TNF-\(\alpha\). Propionibacterium acnes-primed wild-type (WT) mice, but not naive mice, exhibited hyper-coagulation with elevated levels of thrombin–antithrombin complexes and anti-fibrinolytic plasminogen activator inhibitor 1 in their plasma, hypothermia, systemic inflammatory responses and high mortality rate after LPS or TNF-\(\alpha\) challenge. Propionibacterium acnes treatment reportedly induces both Th1 and Th17 cell development. Propionibacterium acnes-primed \(Il12p40^{-/-}\) and \(Ifn\_g^{-/-}\) mice, while not \(Il17A^{-/-}\) mice, evaded all these symptoms/signs upon LPS or TNF-\(\alpha\) challenge, indicating essential requirement of IL-12–IFN-\(\gamma\) axis for the sensitization to LPS and TNF-\(\alpha\). Furthermore, IFN-\(\gamma\) blockade just before LPS challenge could prevent \(P.\) acnes-primed WT mice from endotoxin shock syndrome. These results demonstrated requirement of IFN-\(\gamma\) to the development of endotoxin shock and suggested it as a potent therapeutic target for the treatment of septic shock.

Keywords: hyper-coagulation, hypothermia, sepsis, Th1 cells, TNF-\(\alpha\)

Introduction

Disseminated intravascular coagulation (DIC), a status of hyper-coagulation, is commonly associated with septic shock syndrome (1, 2), in which tumour necrosis factor (TNF-\(\alpha\)) produced by innate immune cells stimulated with pathogen-associated molecular pattern (PAMP) plays a critical role. Intravenous injection of recombinant human TNF-\(\alpha\) activates coagulation system in healthy human and baboon, which is monitored by the elevation of plasma levels of thrombin–antithrombin complexes (TAT) (3, 4). Furthermore, this treatment increases levels of plasminogen activator inhibitor 1 (PAI-1), which promotes a procoagulant status by inhibiting the action of tissue-type plasminogen activator (tPA) to convert plasminogen into fibrinolytic plasmin (3–5). Thus, the balance between PAI-1 and tPA regulates coagulation, and induction of PAI-1 is crucial for the development of prothrombotic state by diminishing plasmin-dependent fibrinolysis. Indeed, plasma PAI-1 levels have been reported to correlate well with the disease severity of septic shock patients with DIC (6–8). We and other investigators reported that formation of intestinal adhesion is also regulated by the balance between PAI-1 and tPA (9, 10). We recently found that the PAI-1 induction is up-regulated by the action of IFN-\(\gamma\) from intestinal NKT cells in post-operative adhesion formation (10). Furthermore, IFN-\(\gamma\) from Th1 cells was reported to be essential for the formation...
of peritonitis-induced intestinal adhesion (11). Thus, it is important to determine whether and how IFN-γ triggers and/or modulates the action of TNF-α to induce hyper-coagulation and other symptoms (12, 13) and high mortality as well (14).

As previously reported, priming with heat-killed Propionibacterium acnes (P. acnes) renders mice highly susceptible to the lethal effects of LPS (15–17). Propionibacterium acnes-primed mice, when challenged with a sub-lethal dose of LPS, develop endotoxin shock syndrome accompanied by high elevation of serum pro-inflammatory cytokines (15, 17, 18). In contrast, P. acnes-primed II12p40+/− mice completely escape from such LPS hyper-responsiveness (19). This strongly suggests the importance of IL-12 and/or IL-23 for P. acnes of TAT and PAI-1 and eventually died of shock. In contrast, mice on a BALB/c background (22) and B6 WT mice were purchased from Clea Japan. We measured TAT concentrations by a commercially available kit from Enzyme Research Laboratories (South Japan). We measured TAT concentrations by a commercially available kit from Enzyme Research Laboratories (South Bend, IN, USA) according to the manufacturer’s instruction.

Core body temperature
Rectal temperature readings were performed using a rectal probe digital thermometer (BAT-10; Physitemp, Clifton, NJ, USA). Difference in rectal temperature post and prior to the challenge was calculated and shown as ΔRectal temperature.

Responsiveness of splenocytes to LPS and TNF-α
Splenocytes (2 × 10^6 ml ^−1) from variously treated mice with various genotypes were incubated with LPS or rTNF-α in vitro. Supernatants were collected for measurement of pro-inflammatory cytokines.

T-cell reconstitution
In total, 2 × 10^7 splenic T cells from naive WT BALB/c mice, enriched by a nylon wool column method (>90% CD3+)(29), were transferred into naive BALB/c nu/nu mice through a tail vein for the T-cell reconstitution, and after 24 h, these T cell-reconstituted nu/nu mice were sequentially administered with P. acnes and challenged with LPS.

Statistics
All data are shown as the mean ± SD of samples in each group. Significance between the experimental and control

**Methods**

**Mice**

II12p40−/− mice on a BALB/c background (22) and II17A−/− mice on a C57BL/6 (B6) background (23) were described elsewhere. II12p40−/− B6 129 mice (24) were backcrossed with BALB/c mice, and F10 mice were used. BALB/c WT, BALB/c nu/nu and B6 WT mice were purchased from Clea Japan (Osaka, Japan). Female mice (8–12 weeks old) were used. All mice were maintained under specific pathogen-free conditions and received human care as outlined in the Guide for the Care and Use of Experimental Animals in Hyogo College of Medicine.

**Reagents**

LPS from Escherichia coli (O55: B5), which selectively activates Toll-like receptor 4 both in vivo and in vitro (25), were purchased from Sigma (St Louis, MO, USA). Heat-killed P. acnes was prepared as described elsewhere (26). Recombinant murine TNF-α was purchased from PeproTech (Rocky Hill, NJ, USA). Hybridoma producing neutralizing anti-IFN-γ mAb (R6A2) was purchased from American Type Culture Collection (ATCC, Livermore, CA). Neutralizing anti-IFN-γ mAb for in vivo treatment was prepared as shown previously (26). The culture medium was RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, 50 µM 2-ME, 2 mM L-glutamine, 100 U ml ^−1 penicillin and 100 µg ml ^−1 streptomycin.

**Sequential administration of P. acnes and LPS or TNF-α**

Mice were administered intra-peritoneally with heat-killed P. acnes (1 mg in 200 µl PBS). At day 5, mice were challenged with various doses of LPS or rTNF-α via a tail vein (26). In some experiments, P. acnes-primed mice were administered intra-peritoneally with various doses of anti-IFN-γ mAb 30 min before LPS challenge (27). At the indicated time points, plasma was sampled for measurement of concentrations of various pro-inflammatory cytokines, TAT and PAI-1 according to the method described by Sommeijer et al. (28). Briefly, anesthetized mice were administered with 180 µl of 3.2% (w/v) sodium citrate via the vena cava, and 10 s later, plasma was sampled through the same syringe. The rectal temperature was periodically monitored. Survival was monitored until 72 and 24 h after challenge with LPS and TNF-α, respectively. We killed all mice that appeared inactive and lost reactions to a supine position and counted them as dead ones.

**Assay for TAT, PAI-1 and cytokines**

An ELISA kit for PAI-1 was purchased from Innovative Research Inc. (Novi, MI, USA). ELISA kits for IFN-γ, TNF-α, IL-6 and IL-12p40 were from R&D (San Diego, CA, USA). HMGB-1 ELISA kits were from SinoTest (Sagamihara-shi, Japan). We measured TAT concentrations by a commercially available kit from Enzyme Research Laboratories (South Bend, IN, USA) according to the manufacturer’s instruction.
groups was examined by the unpaired Student’s t-test. P-values <0.05 were considered significant. Two to three experiments were separately performed, and representative data were shown in each data.

Results

In vivo sensitization to LPS by priming with heat-killed P. acnes

We administered heat-killed P. acnes into WT BALB/c mice and challenged them with LPS (2 or 20 µg per head) at day 5 after this pretreatment. Half of the P. acnes-primed mice died within 12 h after challenge with 2 µg LPS, while all died after challenge with 20 µg LPS (Fig. 1A), indicating that LPS kills the animals in a dose-dependent manner. In sharp contrast, none of naive mice succumbed to these LPS challenges (Fig. 1A). Thus, P. acnes-primed mice are highly susceptible to LPS.

Since hypothermia is an important clinical indicator of sepsis in human (2, 30, 31), we measured rectal temperature of P. acnes-primed mice after LPS challenge. Mice challenged with 2 µg LPS showed 9°C diminution at 6 h and gradually recovered thereafter (Fig. 1B). Mice challenged with 20 µg LPS exhibited 12°C reduction without any recovery. PBS treatment instead of LPS challenge did not affect body temperature or mortality rate of P. acnes-primed mice (Fig. 1A and B).

Elevation of TAT and PAI-1 after LPS challenge

Endotoxin occasionally induces DIC in septic patients (1), and DIC exacerbates septic shock (6–8). Thus, we measured plasma levels of TAT and PAI-1 in P. acnes-primed mice after challenge with 2 µg LPS. Plasma TAT levels were strikingly elevated in P. acnes-primed mice with a peak at 4 h after LPS challenge (Fig. 1C). In contrast, naive mice showed only limited elevation of plasma TAT levels (Fig. 1C). Plasma PAI-1 levels were also dramatically elevated in P. acnes-primed mice after LPS challenge. Compared with the kinetics of TAT induction, PAI-1 level remained at basal levels until 4 h and sharply increased at 8 h after LPS challenge (Fig. 1C). In contrast, plasma PAI-1 levels remained low in naive mice after LPS challenge (Fig. 1C). P. acnes priming alone only modestly increased plasma TAT and PAI-1 levels (Fig. 1C). PAI-1 and tPA mRNA expression

Fig. 1. Increase of the in vivo susceptibility to LPS by priming with heat-killed Propionibacterium acnes. WT BALB/c mice were administered with heat-killed P. acnes. Propionibacterium acnes-primed mice were challenged with 20 or 2 µg of LPS. Survival was monitored until 72 h (A). Rectal temperatures were measured, and difference of rectal temperatures at each time point to that before LPS challenge was shown (B). At various time points after LPS challenge, plasma was sampled for measurement of TAT and PAI-1 concentrations (C) and of IL-6, IL-12p40, TNF-α, IFN-γ and HMGB1 levels (D) by ELISA. A dagger indicates the time point at which all the mice die of shock.
levels increased strikingly (50-fold) and modestly (5.5-fold) in the liver of P. acnes-primed mice after LPS challenge, respectively (Supplementary Figure 1, available at International Immunology Online), suggesting that LPS induces hyper-coagulation status in P. acnes-primed mice by much higher induction of PAI-1 than that of tPA.

Induction of production of pro-inflammatory cytokines by LPS challenge

Propionibacterium acnes-primed mice, but not naive mice, promptly increased plasma levels of IL-6 and TNF-α after LPS challenge (Fig. 1D). They started to increase plasma level of IL-12p40 at 1.5 h, increased it further until 4 h and decreased it rapidly thereafter. We also noticed that they start to increase IFN-γ level at 4 h (Fig. 1D), prior to the increase of PAI-1 level (Fig. 1C). Since high-mobility group box protein 1 (HMGB1) is a potent cytokine that mediates severe sepsis at late stage (32–34), we measured plasma HMGB1 level. Like other pro-inflammatory cytokines, plasma HMGB1 level was dramatically elevated after LPS challenge in P. acnes-primed mice but not in naive mice (Fig. 1D).

Requirement of IL-12p40 for the LPS sensitization

Previously, we demonstrated that P. acnes-primed Il12p40−/− mice showed 100% survival after LPS challenge (19). Thus, we examined whether P. acnes-primed Il12p40−/− mice also evade other symptoms. None of P. acnes-primed Il12p40−/− mice died of endotoxin shock (Fig. 2A) and developed hypothermia (Fig. 2B) after challenge with 2 μg of LPS, which is 50% lethal dose for P. acnes-primed WT mice (Fig. 2A). Furthermore, the elevation of their plasma TAT and PAI-1 levels was very modest (Fig. 2C). This was also the case for TNF-α, IFN-γ or HMGB1 level in the plasma of P. acnes-primed Il12p40−/− mice at 8 h after LPS challenge (Fig. 2D). Taken together, these results indicated that P. acnes-primed Il12p40−/− mice are unresponsive to LPS and strongly suggested that IL-12p40 is necessary for the sensitization to LPS. To verify this possibility, we incubated splenocytes from naive or P. acnes-primed WT and Il12p40−/− mice with LPS and measured pro-inflammatory cytokine levels in their culture supernatants. Compared with those from naive WT mice, splenocytes from P. acnes-primed WT mice produced much larger amounts of TNF-α, IL-6 and IFN-γ upon LPS challenge.

![Graph](https://academic.oup.com/intimm/article-abstract/22/3/157/791307/157)
in vitro. In contrast, splenocytes from *P. acnes*-primed *Il12p40*−/− mice produced small amounts of these cytokines, like as those from naive WT or *Il12p40*+/− mice (Fig. 2E). These results taken together clearly indicated that *Il12p40* is essentially required for *P. acnes*-induced LPS sensitization.

**Importance of Th1 cells but not Th17 cells for the LPS sensitization**

Since *Il12p40*−/− mice failed to develop Th1 cell (Supplementary Figure 2, available at International Immunology Online) and to become sensitized to LPS after treatment with *P. acnes* (Fig. 2A–E), we next investigated whether T cells are required for the sensitization to LPS. As *nu/nu* mice lack thymic T cells, we examined whether *P. acnes* priming is able to induce *nu/nu* mice to be susceptible to LPS. Expectedly, all *P. acnes*-primed *nu/nu* mice could survive after challenge with 20 µg of LPS that could 100% kill *P. acnes*-primed WT mice, suggesting that thymus-derived T cells are required for the *P. acnes*-induced sensitization to LPS (Fig. 3A). To verify this possibility, we transferred WT splenic T cells into *nu/nu* mice and sequentially treated them with *P. acnes* and 20 µg of LPS. All the *nu/nu* mice reconstituted with thymic T cells, like WT mice, became to succumb to the sequential treatment with *P. acnes* and 20 µg of LPS (Fig. 3B). Collectively, these results strongly suggested the importance of T cells for the *P. acnes*-induced sensitization to LPS.

*Il12p40* is a common and essential subunit of IL-12 and IL-23. As IL-23 can activate Th17 cells and as IFN-γ was reported to be capable of sensitizing macrophages to LPS (36), we investigated whether IL-12, like IFN-γ, has the same capacity. To test this, we incubated bone marrow-derived macrophages from WT, *Il12p40*−/− or *Il17A*−/− mice with rIL-12 or rIFN-γ. Then, we stimulated them with LPS. We found that pretreatment with IFN-γ, but not IL-12, is able to enhance production of TNF-α and IL-6 from WT, *Il12p40*−/− or *Il17A*−/− macrophages upon LPS stimulation (Supplementary Figure 3, available at International Immunology Online).

**IL-12 contributes to the sensitization to LPS through induction of IFN-γ**

We next investigated whether Th1 cytokine IFN-γ is essential for the LPS sensitization. Like *Il12p40*−/− mice (Fig. 2), *P. acnes*-primed *Il17A*−/− mice evaded the lethality, hypothermia, hyper-coagulation and systemic inflammation after LPS challenge (Fig. 4A and B). They failed to increase production of TAT, PAI-1, TNF-α and HMGB1 after sequential treatment with *P. acnes* and LPS (Fig. 4C and D). Furthermore, *P. acnes* treatment did not increase the responsiveness to LPS of *Il17A*−/− splenocytes, as illustrated by the failure of splenocytes from *P. acnes*-primed *Il17A*−/− mice to produce large amounts of TNF-α and IL-6 upon challenge with LPS in vitro (Fig. 4E). These results indicated requirement of IFN-γ for the LPS sensitization.

As IFN-γ was reported to be a key cytokine for endotoxin shock (35), we examined possible contribution of IL-17 to the LPS sensitization. Upon LPS challenge, *P. acnes*-primed *Il17A*−/− mice showed survival rate and hypothermia comparable to those of WT mice (Fig. 3C and D). Thus, IL-17 is not profoundly involved in the sensitization to LPS.

**Fig. 3.** Importance of Th1 cells but not Th17 cells for the LPS sensitization. Naive and *Propionibacterium acnes*-primed BALB/c WT (+/+), mice and *nu/nu* mice were administered with various doses of LPS, and mouse survival was monitored until 72 h (A). *nu/nu* mice reconstituted with T cells from WT mice were sequentially treated with *P. acnes* and 20 µg LPS (B). WT B6 mice (black symbols) and *Il17A*−/− B6 mice (blue symbols) were treated with *P. acnes* and subsequently challenged with LPS (C and D). The survival rate after 0.5 or 2 µg LPS (C) and body temperature reductions after challenge with 0.5 µg LPS (D) were monitored until 72 and 8 h, respectively.
Online). Thus, IL-12 lacks the potential to directly sensitize macrophages to LPS either in the presence or in the absence of Il12p40, while IFN-γ could fulfill the potential even in the absence of Il12p40. The results demonstrated prerequisite of IL-12-induced IFN-γ for the P. acnes-induced in vivo sensitization to LPS.

Requirement of IFN-γ for the sensitization to TNF-α

Since P. acnes-primed WT mice are susceptible to exogenous TNF-α (16), we next investigated whether IFN-γ is also critical for the sensitization to TNF-α. Consistent with our previous report (16), P. acnes-primed WT mice showed poor survival after treatment with a sub-lethal dose of TNF-α (Fig. 5A). Moreover, they developed all the symptoms observed in the P. acnes-primed mice with endotoxin shock syndrome (Fig. 5B–D), indicating that TNF-α is capable of replacing LPS in induction of each symptom. TNF-α blockade reportedly can protect against lethal outcome of P. acnes-primed mice after LPS challenge (37). This report together with our present results strongly suggested that TNF-α is a potent effector cytokine involved in the endotoxin shock syndrome. In sharp contrast, P. acnes-primed Ifnγ−/− mice were resistant to the lethal effects of TNF-α (Fig. 5A–D), indicating the importance of IFN-γ for the in vivo sensitization to TNF-α as well. Taken together, these results demonstrated a central role of IFN-γ in the development of the endotoxin shock syndromes via induction of in vivo sensitization to LPS and TNF-α.

IFN-γ also controls LPS challenge phase of endotoxin shock syndrome

We wanted to know whether IFN-γ is also necessary for the development of each symptom or sign during the excitation phase induced by LPS challenge. To test this, we administered neutralizing anti-IFN-γ mAb into P. acnes-primed WT mice at 30 min prior to challenge with 20 μg LPS. Neutralizing anti-IFN-γ mAb could rescue the lethal outcome and the serious hypothermia in a dose-dependent manner (Fig. 6A and B). Neutralizing anti-IFN-γ mAb also prevented the hyper-coagulation (Fig. 6C) and elevation of plasma levels of HMGB1 and TNF-α (Fig. 6D). Thus, IFN-γ is important for the development of each symptom during the excitation phase. Collectively, all the results demonstrated that IFN-γ is a master regulator of the endotoxin shock syndrome.

Discussion

Our present study demonstrated the importance of IL-12–IFN-γ axis for the development of endotoxin shock syndrome. In response to heat-killed P. acnes, macrophages and dendritic cells release IL-12, which induces and activates Tn1 cells (Supplementary Figure 2, available at
Resultant IFN-γ prepares macrophages to be susceptible to LPS, which robustly induces TNF-α production (Fig. 7; Supplementary Figure 3, available at International Immunology Online). Furthermore, P. acnes pretreatment induces mice to be highly susceptible to TNF-α via induction of IFN-γ, which eventually results in their development of hypothermia, hyper-coagulation and lethal shock (Fig. 5). Thus, this IL-12–IFN-γ axis is critical for the in vivo sensitization to both LPS and TNF-α. After LPS challenge, IFN-γ-activated macrophages and perhaps dendritic cells produced large amounts of IL-12 and IL-18 (26), which synergistically activate T<sub>H</sub>1 cells and NK cells to produce IFN-γ (39, 40) (Fig. 7). This IFN-γ positively regulates the development of lethal outcomes, hypothermia, systemic inflammation and hyper-coagulation by strongly increasing responsiveness to LPS and TNF-α and conceivably by synergistically cooperating with LPS and TNF-α (41) (Figs 6 and 7). Accordingly, IFN-γ is a central cytokine that initiates both the hypersensitization to LPS during P. acnes priming phase and the development of endotoxin shock syndrome after LPS challenge (Fig. 7).

This study does not exclude roles of NK cells as a cell source of IFN-γ during the priming and effector phases. However, we found that P. acnes-primed <i>nu/nu</i> mice are resistant to 20 μg of LPS, which kills 100% P. acnes-primed WT mice, and that reconstitution with WT T cells provided <i>nu/nu</i> mice with the capacity to develop LPS susceptibility after P. acnes treatment (Fig. 3A and B), indicating the importance of T<sub>H</sub>1 cells for LPS sensitization. However, we also found that P. acnes-primed <i>nu/nu</i> mice died of endotoxin shock after challenge with high dose of LPS (200 μg per head) (Fig. 3A), suggesting possible contribution of NK cell production of IFN-γ to the LPS sensitization. Therefore, in the <i>nu/nu</i> mice NK cells might, at least partly, participate in the establishment of P. acnes-induced sensitization to LPS by production of IFN-γ in response to IL-12 and IL-18.

IL-18 is also a potent IFN-γ-inducing cytokine. However, in contrast to Il12p40<sup>−/−</sup> mice, Il18<sup>−/−</sup> mice shows normal susceptibility to the sequential treatment with P. acnes and LPS (19). This is partly due to the facts that IL-18 has little capability to induce T<sub>H</sub>1 cell development (40) and that IL-18 does not affect IL-12 production (42). Thus, IL-12 is critically involved in the P. acnes-induced sensitization to LPS by production of IFN-γ in response to IL-12 and IL-18.

Our present study revealed the importance of IFN-γ even during the excitation phase induced by LPS challenge. IFN-γ blockade 30 min prior to LPS challenge protected against all the endotoxin shock-associated alterations (Fig. 6). Several mechanistic possibilities might explain the involvement of IFN-γ in the development of TNF-α-mediated endotoxin...
shock syndrome. First, IFN-γ might modulate production of TNF-α after LPS challenge (Fig. 4E; Supplementary Figure 3, available at International Immunology Online). IFN-γ was reported to enhance TNF-α production induced by LPS (41). In fact, IFN-γ blockade significantly hampered plasma increase of TNF-α after LPS challenge (Fig. 6D). Second, although acting on the very late phase of P. acnes priming, this IFN-γ blockade may be able to desensitize the established sensitivity to LPS in P. acnes-primed WT mice. Third, IFN-γ and TNF-α might synergize for the development of each symptom. It is well established that IFN-γ and TNF-α synergize for production of various cytokines/chemokines, exemplified by IL-6 and CXCL10 (IP-10), via activating nuclear factors, such as STAT-1/IFN regulated factor 1 and NF-κB (41, 43). Likewise, the cooperative activation of these nuclear factors might control production of key factors involved in the development of each symptom.

HMGB1, originally discovered as a nuclear protein, was recently reevaluated as a potent late phase mediator of severe sepsis (32–34). HMGB1 levels are reported to elevate during severe sepsis in humans and animals. Furthermore, HMGB1 blockade prevents septic animals from lethality. We demonstrated that LPS induces an increase in the plasma level of HMGB1 in an IFN-γ-dependent manner (Figs 4D and 6D). As it is capable of inducing production of pro-inflammatory cytokines and chemokines in inflammatory cells, HMGB1 might be another potent target for the treatment of endotoxin shock syndrome.

PAI-1-induced hyper-coagulation seems to be beneficial for host defense against local bacterial invasion. Bacteria have unique proteolytic machinery for their successful invasion into mammalian host. Most important proteolytic proteins are plasminogen activators. Individual bacteria produce their own plasminogen activators, exemplified by streptokinase and staphylokinase produced by Group A Streptococcus pyogenes and Staphylococcus aureus, respectively. Bacterial plasminogen activators destroy host extracellular matrix barrier, allowing them to invade deeper into the host and finally to establish their infection (44). However, PAI-1-induced fibrin deposition surrounding the initial invasion sites might enclose the destroyed extracellular matrix and protect against bacterial translocation and dissemination by serving as a new barrier, eventually strengthening the efficient bacterial eradication. Therefore, local hyper-coagulation induced by IFN-γ might be regarded as a potent host defense weapon. In other word, immune cells produce IFN-γ, which protects host tissue from bacterial invasion by encapsulating them with thrombus. Thus, PAI-1 might be a potent host defense molecule induced by inflammatory and Th1 responses. Indeed, Yai1−/− mice are highly susceptible to pneumonia induced by airway infection with Klebsiella pneumoniae, a Gram-negative bacterium (45).

IFN-γ and TNF-α are essential for host defense against various pathogens by activating phagocytes and inducing inflammation. Upon microbial infection, mammalian host produces appropriate amounts of these cytokines

**Fig. 6.** IFN-γ profoundly controls endotoxin shock syndrome. Propionibacterium acnes-primed WT BALB/c mice were administered with various doses of neutralizing anti-IFN-γ 30 min prior to LPS challenge. Survival rate (A) and rectal temperature decrease (B) were monitored. At 4 h (open columns) or 8 h post LPS challenge, plasma was sampled for analysis of hyper-coagulation (TAT and PAI-1 concentrations) (C) and for measurement of HMGB1 and TNF-α concentrations (D).
IFN-γ is a key cytokine for endotoxin shock

Supplementary data

Supplementary data are available at International Immunology Online.

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References


under the proper control of cytokine activation cascades. For example, following Listeria monocytogenes infection, mice produce various pro-inflammatory cytokines, including TNF-α, IL-12 and IL-18, via recognizing listerial PAMPs by pattern recognition receptors such as TLR and Nod-like receptor (46, 47). IL-12 and IL-18 then induce production of IFN-γ, which in collaboration with TNF-α efficiently eliminates L. monocytogenes. Thus, appropriate amounts of IFN-γ and TNF-α are beneficial for the host. However, dysregulated production of or responsiveness to those cytokines often leads to diseases, exemplified by the endotoxin shock syndrome. Thus, IFN-γ might tip the balance of actions of TNF-α. Furthermore, IFN-γ primarily contributes to the development of severe liver injury induced by activation of a second cell death receptor Fas, as well. Propionibacterium acnes-primed mice, but not naive mice, develop massive liver injury after challenge with soluble Fas ligand (48), while P. acnes-primed Ifn-γ−/− mice can evade this injury (our unpublished data). Thus, IFN-γ might play a central role in the development of severe illnesses and syndromes that are caused by activation of cell death receptors.

Endotoxin shock is a life-threatening condition. Thus, it is very important to determine the master regulator of endotoxin shock. Our present study could reveal that IFN-γ is a master regulator of endotoxin shock and neutralization of IFN-γ even just before LPS challenge could rescue animals from endotoxin shock. Many investigators revealed the molecular mechanisms how IFN-γ synergizes with LPS and/or TNF-α for induction of various gene expressions in vitro (41). However, it is still to be elucidated how endogenous IFN-γ synergizes with LPS and TNF-α for in vivo induction of hypothermia, hyper-coagulation and shock. Although we need extensive efforts to resolve this issue, we believe our data present key information on the treatment of endotoxin shock syndrome.

Fig. 7. A proposal model for the endotoxin shock in Propionibacterium acnes-primed mice. After priming with heat-killed P. acnes, macrophages produce IL-12, which causes T h1 cell development. IFN-γ produced by the T h1 cells prime macrophages to be highly susceptible to LPS. Besides, IFN-γ renders mice highly susceptible to TNF-α. After challenge of P. acnes-primed mice with LPS, IFN-γ-primed macrophages produce robust IL-12 and IL-18, which then activates T h1 cells and NK cells to produce a large amount of IFN-γ. The IFN-γ-primed macrophages simultaneously produce enormous TNF-α. TNF-α, in turn, might act on the cells that become highly responsive to TNF-α after P. acnes priming to initiate the development of hypothermia, hyper-coagulation, systemic inflammatory responses and lethal shock. IFN-γ positively regulates the development of those clinical symptoms and signs. Thus, IFN-γ is a central factor that primarily controls both P. acnes-induced priming phase and the effector phase induced by LPS challenge.


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