Interleukin-10 is a central regulator of the response to LPS in murine models of endotoxic shock and the Shwartzman reaction but not endotoxin tolerance.

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Interleukin-10 is a Central Regulator of the Response to LPS in Murine Models of Endotoxic Shock and the Shwartzman Reaction but not Endotoxin Tolerance

Daniel J. Berg,* Ralf Kühn,† Klaus Rajewsky,‡ Werner Müller,§ Satish Menon,‖ Natalie Davidson,* Gabriele Grünig,* and Donna Rennick*  
*Department of Immunology and †Molecular Biology, DNAX Research Institute of Cellular and Molecular Biology, Palo Alto, California 94304; and ‡Institute for Genetics, University of Cologne, Cologne, Federal Republic of Germany

Abstract

Previous studies in vivo have shown that IL-10 infusion can prevent lethal endotoxic shock. Mice deficient in the production of IL-10 (IL1OT) were used to investigate the regulatory role of IL-10 in the responses to LPS in three experimental systems. In a model of acute endotoxic shock, it was found that the lethal dose of LPS for IL1OT mice was 20-fold lower than that for wild type (wt) mice suggesting that endogenous IL-10 determines production of experimental it was 20-fold lower than that for wild type (wt) mice suggesting that endogenous IL-10 determines the amount of LPS which can be tolerated without death. The high mortality rate of IL1OT mice challenged with modest doses of LPS was correlated to the uncontrolled production of TNF as treatment with anti-TNF antibody (Ab) resulted in 70% survival. Additional studies suggested that IL-10 mediates protection by controlling the early effectors of endotoxic shock (e., TNFα) and that it is incapable of directly antagonizing the production and/or actions of late appearing effector molecules (e., nitric oxide). We also found that IL1OT mice were extremely vulnerable to a generalized Shwartzman reaction where prior exposure to a small amount of LPS primes the host for a lethal response to a subsequent sublethal dose. The priming LPS dose for IL1OT mice was 100-fold lower than that required to prime wt mice implying that IL-10 is important for suppressing sensitization. In agreement with this assumption, IL-10 infusion was found to block the sensitization step. Interestingly, IL-10 was not the main effector of endotoxin tolerance as IL1OT mice could be tolerated to LPS. Furthermore, IL-10 infusion could not substitute for the desensitization dose of LPS. These results show that IL-10 is a critical component of the host’s natural defense against the development of pathologic responses to LPS although it is not responsible for LPS-induced tolerance. (J. Clin. Invest. 1995, 96:2339–2347.) Key words: IL-10 • endotoxic shock • Shwartzman reaction • endotoxin tolerance

Introduction

Severe gram-negative bacterial infections can lead to the development of endotoxic shock, a state characterized by hypoten-
travascular coagulation and shock) (21, 22), and acquired endotoxin tolerance (23, 24).

Methods

Animals. IL10T mice generated on a C57Bl/6/129-Ola background (20) and wt litter-mate controls were derived by cesarean at Simonsen Laboratory (Gilroy, CA) and maintained in our animal facility at DNAx (Palo Alto, CA). Healthy 4–5 wk old IL10T mice were used for this study.

Reagents. LPS from Escherichia coli (serotype O111:B4) was obtained from DIFCO (Detroit, MI) and resuspended in pyrogen-free saline. Recombinant murine IL-10 was expressed in E. coli as inclusion bodies and purified after renaturation to homogeneity and high sp act (\(~1.0 \times 10^7 \text{U/mg}\)) using hydrophobic and ion exchange chromatography. The protein concentration in the purified preparations was determined by the extinction coefficient of the protein (1 mg/ml = 0.36 A280). This material contained < 0.10 EU/mg protein, and remained stable at 4°C for at least 6 mo. The specific activity of murine IL-10 was evaluated in a proliferation assay using Ba/F3 cells carrying the IL-10 receptor (25). Recombinant IL-10 was diluted in saline and a dose of 10 \(\mu\)g/mouse, i.p., was administered in a total volume of 250 \(\mu\)l.

Determination of serum cytokine and NO levels. Blood samples were obtained by cardiac puncture from mice euthanized with CO2. Serum concentrations of TNF\(\alpha\) and IL-1\(\alpha\) were measured using ELISA kits purchased from Genzyme (Boston, MA) as per the manufacturer’s directions. Other serum cytokine levels were detected by two-site sandwich ELISA assays as previously described (26), using the following antibody pairs which were kindly provided by Dr. J. Abrams (DNAx): for IFN\(\gamma\), R46A2; and AN18; for IL-6, 32C1, and 20F3; and for IL-10, SXC-11, and XT3. The antibodies to the p40 subunit of IL-12, C15.1.2 and C15.6.7 (27) were kindly provided by Anne O’Garra (DNAx). Biological activity of the IL-12 heterodimer was quantified by an Ab capture bioassay as previously described (28). Murine rIL-12 purified from the supernatant of COS 7 cells transfected with the cDNAs encoding the p35 and p40 subunits of IL-12 was kindly provided by Dr. M. Bond, DNAx. Serum nitrate levels were measured by reducing nitrate enzymatically with nitrate reductase (29). Serum was diluted 1:5 in water and the enzyme reaction was performed using a kit purchased from Boehringer-Mannheim (Indianapolis, IN) as per the manufacturer’s directions.

Experimental protocol for induction of endotoxic shock. Mice were injected i.v. via the tail vein with LPS (2.5, 5, 10, 50, 100, or 200 \(\mu\)g/mouse) in a saline solution of 200 \(\mu\)l per dose and monitored for survival for 7 d. All deaths occurred within 3 d of LPS administration. All protocols were reviewed and approved by the institute’s (DNAx) animal care committee.

Treatment with anti-cytokine antibodies. WT and IL10T Mice were injected intraperitoneally with various concentrations of purified rat anti-mouse neutralizing anti-cytokine Abs or isotype control Abs (5, 10, 10 mg). These Abs were administered either 4 h before, concurrent with or 2, 4, or 6 h after LPS administration as indicated in the result’s section. Antibodies used herein were: anti-IL-10 (JESS-2A5); anti-IL-6 (20F3); anti-TNF\(\alpha\) (XT22); anti-IFN\(\gamma\) (XM112); and anti-\(\beta\)-galactosidase (GL-117) which is referred to as the isotype control. The anti-TNF\(\alpha\) antibody, XT22, was elicited against TNF\(\alpha\) but neutralizes both TNF\(\alpha\) and TNF\(\beta\). The antibody concentrations used herein have been shown to block the adverse affects of LPS in wt mice (when possible).

The highest concentrations used (10 mg/mouse) were 2- to 5-fold higher than those required to ablate the adverse affects of endogenously produced cytokines in normal mice in a model of LPS-induced toxic shock (19) or in other in vivo models (30, 31, 32).

Experimental protocol for the generalized Shwartzman reaction and endotoxin tolerance. The generalized Shwartzman reaction and endotoxin tolerance were elicited in mice by two consecutive injections of E. coli LPS. A preparatory injection was given in varying doses in the f.p. followed 24 h later by an i.v. provoking dose (elicitation). The preparatory dose for the Shwartzman reaction in IL10T mice was 0.005 to 0.01 \(\mu\)g LPS; in wt mice the dose was 1 \(\mu\)g. The provoking dose for the IL10T mice was 2.5 \(\mu\)g; for wt mice the dose was 100 \(\mu\)g. The preparatory dose for endotoxin tolerance in IL10T mice was 1.0 \(\mu\)g; in wt mice the dose was 25 \(\mu\)g. The provoking dose for the IL10T mice was 5–10 \(\mu\)g; for wt mice the dose was 200 \(\mu\)g. In some experiments, serum was obtained at varying intervals after LPS injection (provoking dose) for measurement of cytokine levels, in the manner described above.

Statistical analysis. Significant differences between experimental groups were evaluated by the nonparametric Mann and Whitney U test.

Results

IL10T mice show increased susceptibility to endotoxic shock. IL10T and wt mice were injected with the indicated doses of LPS i.v. and monitored for survival for 7 d. Each treatment group consisted of 6–8 wt or IL10T mice. Results are expressed as percentage of survival and are representative of three independent experiments.

Figure 1. IL10T mice have increased susceptibility to endotoxic shock. IL10T and wt mice were injected with the indicated doses of LPS i.v. and monitored for survival for 7 d. Each treatment group consisted of 6–8 wt or IL10T mice. Results are expressed as percentage of survival and are representative of three independent experiments.

<table>
<thead>
<tr>
<th>WT mice</th>
<th>IL10T mice</th>
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<tr>
<td>200 (\mu)g LPS</td>
<td>2.5 (\mu)g LPS</td>
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<tr>
<td>(\leq 100) (\mu)g LPS</td>
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<td>100 (\mu)g LPS</td>
<td>100 (\mu)g LPS</td>
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</tbody>
</table>

In the wt mice, serum TNF\(\alpha\) levels 3-6 fold higher than that observed in IL10T mice.

Cytokine levels in the sera of IL10T and wt mice were measured at various intervals after LPS administration. The dose of LPS used for this experiment was 50 \(\mu\)g/mouse as it produced 100% mortality in IL10T mice and no mortality in wt mice (refer to Fig. 1). It was found that TNF\(\alpha\) levels were greatly increased in IL10T mice as compared with wt mice (Fig. 2 A). At 1 h post LPS challenge, the TNF\(\alpha\) levels were identical in the IL10T and wt mice. In the wt mice, serum TNF\(\alpha\) declined to almost undetectable levels 3–6 h after LPS. However, TNF\(\alpha\) continued to increase in the sera of IL10T mice and reached a peak concentration at 3 h that was 30-fold higher than the amount detected in wt mice at 3 h. These high levels of TNF\(\alpha\) were maintained in the sera of IL10T mice at
6 h after LPS. Thus, both the kinetics and level of TNFα production were markedly altered in the absence of endogenous IL-10.

Similar results were observed when the levels of IL-6 and IL-12 were measured (Fig. 2 B and C). As expected, the appearance of IL-6 and IL-12 in the sera of IL10T and wt mice occurred later than that of TNFα. While the levels of these two cytokines declined in wt mice after 3 h, they continued to rise in IL10T mice. At 6 h post LPS, IL-6, and IL-12 were abnormally increased in IL10T mice by 70- and 6-fold, respectively. Additionally, p40 (the inducible subunit of IL-12), IL-1α (Fig. 2 D) and IFNγ (Fig. 2 E) were much higher in the sera of IL10T mice.

Serum nitrate could not be detected in the sera of IL10T or wt mice before or within 6 h of LPS administration (data not shown). However, very high levels were detected in the sera of IL10T mice at 12 h (data not shown) and 18 h post LPS administration (Fig. 2 E). In contrast, nitrate remained undetectable in the sera of wt mice at all time points tested (Fig. 2 E).

**TNF and IFNγ are mediators of mortality in IL10T mice challenged with LPS.** IL10T mice were infused with neutralizing anti-cytokine Ab in order to determine which cytokine(s) was contributing to their high mortality rate after challenge with 50 μg of LPS, a dose which was sublethal for wt mice. In these experiments, 2 mg of anti-TNF Ab administered 4 h before LPS challenge resulted in 70% survival whereas administration of 2 mg of the isotype control Ab provided no protection (Fig. 3).

Under the same experimental conditions, partial protection of IL10T mice (36% survival) was observed by administering

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*Figure 2. IL10T mice have dysregulated cytokine production after stimulation with endotoxin. LPS (50 μg/mouse) was administered i.v. and sera collected at the indicated time points. Each data point represents the mean±S.D. of 6–8 individual mice per group. (A) TNFα, (B) IL-6, (C) IL-12 bioactivity and p40 protein concentration, (D) IL-1α, and (E) IFNγ (left) and serum nitrate (right). Results are representative of two independent experiments. * Mann-Whitney U test, P < 0.05, demonstrating statistical difference in serum levels between IL10T and wt mice groups. ND, not detected.*
at least 10 mg of anti-IFNγ Ab. Lower amounts of anti-IFNγ (i.e., 2 mg) extended the survival of these mice but did not ultimately prevent their death. The diminished mortality observed in LPS-challenged IL1OT mice treated with the high dose of anti-IFNγ Ab demonstrates that this cytokine contributes to their mortality. In contrast, the administration of anti-IL-6 Ab (2–10 mg) failed to protect IL1OT mice from the lethal effects of LPS (Fig. 3). Similar results were observed in wt mice challenged with LPS (data not shown).

Endogenously produced IL-10 regulates early events in endotoxic shock. Kinetic studies of IL-10 production were performed in order to further analyze the role of this cytokine in the regulation of the host’s response to endotoxin. After challenge of wt mice with LPS (200 μg), it was found that serum IL-10 levels peaked within 2 h of challenge (Fig. 4 A) and remained detectable at 6 h. IL1OT mice challenged with LPS did not produce detectable amounts of IL-10 in agreement with previous studies (20). The ability of endogenous IL-10 to protect the host against the development of lethal endotoxic shock was evaluated by administering anti–IL-10 Ab to wt mice at different time points with respect to a sublethal LPS challenge (50 μg/mouse). Neutralizing Ab to IL-10 administered 4 h before LPS injection reduced the survival of the wt mice to 10% as compared with 100% survival in the isotype control-treated group (Fig. 4 B). If the administration of the anti–IL-10 Ab were delayed until 3 or 6 h after LPS, 90 and 100% survival, respectively, were observed. These results indicate that the events regulated by endogenous IL-10 occur early in endotoxic shock.

As IL1OT mice are deficient in the production of IL-10, they were infused with exogenous IL-10 (10 μg) at various times relative to a lethal dose of LPS in order to further define IL-10’s regulatory effects. If IL-10 infusion were given at the same time as LPS (50 μg), 100% of the IL1OT mice survived. However, if IL-10 infusion were delayed even 2 h after LPS challenge, no survival was observed. Identical results were obtained when a fivefold lower, but still lethal, LPS dose (10 μg) was used (data not shown). These results confirmed that IL-10 must act early in order to block the pathogenic effects of LPS. Our results also show that 90% of the IL1OT mice survived when IL-10 was administered 4 h prior to LPS challenge. This outcome suggested that IL-10 persisted in vivo long enough to directly antagonize the actions of LPS or that IL-10 invoked a state of unresponsiveness in LPS-sensitive cells. This latter possibility was addressed in the studies described below.

The regulatory effects of IL-10 in the generalized Shwartzman reaction. The development of the generalized Shwartzman reaction was compared in wt and IL1OT mice in order to evalu-
Figure 5. IL10T mice can be sensitized to LPS in the generalized Shwartzman reaction. The Shwartzman reaction was elicited in IL10T mice by two consecutive injections of LPS. The preparatory injection (0.005-0.1 μg LPS, f.p.) was followed 24 h later by a standard 2.5 μg i.v. provocating dose. Each group consisted of 6-8 IL10T mice. Data are representative of 3 independent experiments and results are expressed as percentage of survival.

ate the role of endogenously produced IL-10 in controlling this type of LPS-induced response. Extremely small amounts of LPS (0.001-0.005 μg LPS) injected into the f.p. of IL10T mice primed them for a lethal reaction to a sublethal dose of LPS given i.v. 24 h later (Fig. 5). In contrast, a minimum dose of

1 μg LPS was required to sensitize a wt mice for a lethal reaction to a sublethal dose of LPS (data not shown). To further evaluate this phenomenon, serum cytokines were measured at various intervals after administration of the LPS elicitation dose. Prior LPS sensitization of IL10T mice lead to a transient but significant increase in TNFα production (Fig. 6 A). In fact, the highest amount of TNFα induced after two consecutive non-lethal doses of LPS was similar to that induced by a single lethal dose (see Fig. 2). In contrast, IL-6 and IFNγ serum levels were not significantly altered by priming IL10T mice with LPS (Figs. 6, B and C). In both cases, the levels observed were low as compared to those elicited by a single lethal dose of LPS (see Fig. 2).

In another set of studies, we tested the ability of exogenous IL-10 to regulate the priming phase of the generalized Shwartzman reaction. IL10T and wt mice were given either IL-10 or saline simultaneously with the priming dose of LPS (Table 1). As expected, we found that IL10T and wt mice primed with LPS had high mortality rates when challenged with sublethal doses of LPS. In contrast, the mortality rates of both IL10T and wt mice were dramatically reduced if they were primed with LPS in the presence of IL-10. These results clearly demonstrate that the priming step in the Shwartzman reaction is inhibitable by IL-10.

**IL10T mice develop endotoxin tolerance.** Numerous studies have shown that prior exposure to low doses of LPS can result in the development of tolerance to subsequent LPS challenge. The possibility that the induction of tolerance might depend on the actions of IL-10 prompted us to test whether IL10T mice could be rendered tolerant to LPS. When IL10T mice were primed with 1 μg of LPS in the f.p. and subsequently challenged with a lethal i.v. dose of LPS (5 μg), all of the mice survived (Fig. 7). IL10T mice primed with 1 μg of LPS survived subsequent challenges of 5-10 μg of LPS; however no survival was seen when the mice were challenged with 25 μg of LPS (data not shown). Serum cytokines were measured at various inter-

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**Figure 6.** IL10T mice sensitized to LPS in the Shwartzman reaction have exaggerated production of TNFα. IL10T mice received either saline or 0.005 μg LPS as a preparatory dose in the f.p. followed 24 h later by an eliciting dose of 2.5 μg LPS i.v. Sera for cytokine measurement by ELISA were obtained at the indicated time points. Data represented are the mean±S.D. of 6-8 individual mice per group. (A) TNFα, (B) IL-6, and (C) IFNγ. * Mann-Whitney U test, P < 0.05, demonstrating statistical difference in the levels between LPS-primed and saline-treated IL10T mice groups.
The generalized Schwartzman reaction was elicited by two consecutive injections of E. coli LPS. The preparatory injection i.p. (wt, 1 μg LPS; IL1OT, 0.005 μg LPS) followed after 24 h by an i.v. eliciting dose (wt, 100 μg LPS; IL1OT, 2.5 μg LPS). Saline or IL-10 (10 μg) was administered i.p. to the indicated groups. (−) indicates the administration of saline. Data are pooled from two independent experiments.

Next we evaluated the effect of exogenous IL-10 in the development of endotoxin tolerance in wt and IL1OT mice (Table II). It was found that IL-10 could not substitute for the LPS priming dose, as no tolerance to the eliciting LPS dose could be demonstrated. When IL-10 and the priming LPS dose were administered simultaneously, the induction of tolerance was not antagonized by IL-10. In fact, IL-10 administration appeared to enhance the tolerizing effects of LPS in both wt and IL1OT mice.

Discussion

We have used IL1OT and wt mice to further define the regulatory role of endogenous IL-10 in the immune/inflammatory responses elicited in vivo by LPS. In a model of acute endotoxemia, our comparative studies showed that the amount of LPS which induced lethal endotoxic shock in IL1OT mice was at least 20-fold lower than that required by wt mice. These results suggest that the host’s endogenous IL-10 level determines the amount of endotoxin which constitutes a lethal threshold dose. In support of this conclusion, it was found that infusing IL1OT mice with 10 μg of IL-10 enabled them to survive a 10-fold higher dose of LPS (50 μg) than their untreated littermates. Presumably, the administration of small doses of recombinant IL-10 (0.5–1.0 μg) used to protect normal mice from lethal doses of LPS (16, 17) reflects the added protection gained by boosting their endogenous IL-10 levels.

The high mortality rate observed in IL1OT mice challenged with LPS was correlated with greatly elevated levels of serum TNFα and IFNγ. The heightened production of these two cytokines in response to LPS had been observed in mice rendered IL-10–deficient by anti–IL-10 Ab (19). Herein, high levels of serum IL-1α, IL-6, IL-12, and nitrate were also detected in the sera of LPS-challenged IL1OT mice. Wt mice which survived the same dose of LPS produced low, transient levels of serum cytokines and no detectable nitrate. Although it was shown previously that IL-10 was capable of suppressing the in vitro production of these various pro-inflammatory mediators (12, 13), our studies with IL1OT mice and those using neutralizing Ab (19) have demonstrated that endogenous IL-10 is essential for controlling their in vivo production.

Even though a bolus i.v. injection of LPS induced the exaggerated production of multiple pro-inflammatory mediators in IL-10–deficient mice, significant protection was provided by prior treatment with anti-TNF Ab. These preliminary results suggested that the uncontrolled production of TNF plays an important role in the increased susceptibility of IL1OT mice to endotoxic shock. It is well known that TNFα not only causes tissue necrosis and organ failure, but is also a potent inducer of the enzyme nitric oxide synthase which is required for the production of nitric oxide (33). Nitric oxide has been implicated in the induction of hypotension and vascular unresponsiveness (34–36) associated with endotoxic shock. Because elevated serum nitrate levels were observed in IL1OT mice but were absent in normal mice stimulated with the same dose of LPS, it seems likely that the unregulated production of nitric oxide contributes to the high mortality rates observed in the IL1OT mice. The exaggerated production of nitric oxide in response to such low levels of LPS may reflect the absence of IL-10’s regulatory effects on TNFα production.

Others have used a similar model of endotoxic shock to show that the survival rates of normal mice challenged with lethal doses of LPS were improved substantially by the administration of anti–IFNγ Ab (6, 10). Conversely, survival rates of normal mice challenged with a LD50 of E. coli were greatly diminished if the mice were pretreated with IFNγ (6). Furthermore, high mortality rates were observed in normal mice treated with a combination of IFNγ and TNFα at doses that were nonlethal when administered alone (6). These latter studies were intriguing since they suggested that IFNγ may play a detrimental role by augmenting the pathogenic effects of TNFα. The improved survival observed in LPS-challenged IL1OT mice treated with anti-IFNγ Ab supports the premise that IFNγ contributes to endotoxic shock. Although we did not use anti–IL-1 Ab or IL1 receptor antagonist (IL-1RA) in this study, it has been shown that these agents can prevent lethality in animal
models of sepsis (8, 9, 37). Clearly toxic shock is mediated by a complex set of cytokine interactions (2–10). Thus, the lethal threshold dose of LPS for IL10T mice may be extremely low as compared to that for WT mice because IL10T mice are incapable of regulating not only TNFα production but also the production of other mediators which may function as amplifiers (i.e., IFNγ and IL-1) or as effectors of endotoxic shock.

As we and others (18, 19) have found that IL-10 appears in the sera of normal mice within 1 h after LPS challenge and persists in their blood for at least 6 h, we investigated the possibility that IL-10 may be important for directly suppressing late as well as early events leading to endotoxic shock. Our preliminary experiments confirmed that treatment of WT mice with anti–IL-10 Ab prior to challenge with a sublethal dose of LPS resulted in high mortality rates (19). We also observed decreased survival in WT mice given anti–IL-10 Ab at the same time as LPS but not when the Ab-treatment was delayed until 3 h after challenge. Based on the results of these in vivo kinetic studies, it appeared that IL-10 mediates protection in the earliest phase of the LPS response. In our studies with IL10T mice, the importance of IL-10 in controlling early and late events was investigated by administering IL-10 at different times relative to a low LD_{100} (10 μg) and a high LD_{100} (50 μg) of LPS. We found that IL10T mice were completely protected if IL-10 were administered just prior to or at the same time as LPS. The failure of IL-10 to protect mice when administered even two hours after challenge with either a low or high LD_{100} further demonstrates that IL-10 plays a critical role in regulating early events such as TNFα production. The pathological effects of late appearing mediators, such as IFNγ and nitric oxide, are most likely diminished as a consequence of IL-10’s suppressive actions on TNFα production.

We have also investigated whether IL-10 plays a regulatory role in the generalized Shwartzman reaction (21, 22). This reaction requires two consecutive, relatively low doses of LPS and is clinically manifested as a shock syndrome. Because the amount, route and timing of the LPS injections are critical for the phenomenon to occur, it was proposed that the Shwartzman reaction is dependent on a cascade of inflammatory mediators (38–40). Recently, it has been shown that the first dose of LPS induces the production of IL-12 (41). IL-12 subsequently stimulates the production of IFNγ which then prepares macrophages and other cell types to release large amounts of inflammatory cytokines in response to the second, provoking dose of LPS (41). It appears that TNFα is the critical effector of lethality in primed mice but its actions require the presence of either IL-10 or IFNγ (39, 41). After recognizing that the sequential actions of very specific cytokines are involved in generating the Shwartzman reaction (41) and based on what was known about the in vitro kinetics of IL-10 production (12), it was

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**Table II. IL-10 Cannot Substitute for the LPS Priming Step in Tolerance**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>IL-10 treatment</th>
<th>LPS priming</th>
<th>LPS elicitation</th>
<th>No. of mice alive/ tested</th>
<th>Survival</th>
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<td>f.p., 9 h</td>
<td>i.v., 24 h</td>
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</table>

Tolerance to LPS was elicited by two consecutive injections of E. coli LPS. The preparatory f.p. injection (wt, 25 μg LPS; IL10T, 1 μg LPS) was followed 24 h later by an i.v. eliciting dose of LPS (wt, 200 μg LPS; IL10T, 10 μg LPS). Saline or IL-10 (10 μg) was administered i.p. to the indicated groups. (—) indicates the administration of saline. Data are pooled from two independent experiments.
predicted (41) that any putative inhibitory effects of endoge-
nous IL-10 would occur only during the second LPS challenge.
However, our comparative studies have shown that only minis-
cule amounts of LPS were required to prime IL10T mice for the 
enhanced release of TNFα. The finding that 200-fold more 
LPS was needed to prime wt mice suggests that endogenous 
IL-10 normally inhibits IL-12 and IFNγ production in response 
to small amounts of LPS during the preparatory phase.

In support of this conclusion, we formally demonstrated that 
IL-10 is capable of blocking LPS priming in the Shwartzman 
reaction. Hence, IL-10T and wt mice primed with LPS together 
with IL-10 were protected from a lethal provoking LPS dose. 
When cytokine levels in the serum of primed and unprimed 
IL10T mice were compared, no differences were observed in 
their IL-6 or IFNγ production after LPS challenge. However, 
elevated TNFα levels were found in the primed mice further 
indicating that alterations in TNFα production are a necessary 
prerequisite for a lethal Shwartzman reaction (39, 41). These 
results also support the conclusion that IL-10 normally sup-
presses the host’s reactions to minimal amounts of LPS thus 
preventing increased susceptibility to systemic shock.

Tolerance to endotoxin can develop in animals exposed re-
peatedly to sublethal doses of LPS or exposed to a single low 
dose of LPS albeit higher than that used to prime for the 
Shwartzman reaction (23). Tolerance occurs rapidly (24–96 
h) and has been correlated with the suppressed production of 
inflammatory mediators (23, 39, 42). It has been proposed 
that hyporesponsiveness may result from the induction of both 
inflammatory and anti-inflammatory factors following exposure 
to LPS. The supernatants of macrophages tolerized in vitro to 
LPS have been shown to contain a factor capable of rendering 
naive macrophages refractory to LPS stimulation (43). We have 
tested the possibility that IL-10 is the factor responsible for LPS-
duced tolerance. The results of our studies clearly suggest 
otherwise since certain doses of LPS could establish a state of 
hyporesponsiveness in IL10T mice. As a result, the survival 
rate upon lethal LPS challenge was 87% as compared with 0% 
in the untolerized group. Evaluation of cytokines in the sera of 
tolerized IL10T mice showed that protection correlated with 
suppressed TNFα production; no alterations were seen in either 
IL-6 or IFNγ production.

A potential role for IL-10 in establishing LPS-induced toler-
ance was further evaluated by administering exogenous IL-10 
to IL10T and wt mice. Herein, it was shown that IL-10 infusion 
could not substitute for LPS in eliciting tolerance since none of 
the IL-10–treated animals survived a subsequent lethal LPS 
challenge. Nevertheless, the injection of IL-10 together with 
LPS during the desensitization step resulted in a slight enhance-
ment of the survival rate (100%) over that obtained with just 
LPS (72 and 87%). Collectively, our results show that the 
mechanism of LPS-induced tolerance is not dependent on 
IL-10, although IL-10 may serve to potentiate the process. Several 
anti-inflammatory factors are produced in response to LPS and 
may mediate acquired tolerance to endotoxin. Examples include 
the IL-1RA (44) and IL-6 (45). Other possibilities include 
modulation of the LPS receptor or post-receptor events after 
LPS stimulation (46, 47) as well as the production of the protein 
BPI (bactericidal/permeability-increasing protein) from LPS-
activated PMNs (48). It is interesting to note that the production 
of one of these anti-inflammatory agents, the IL-1RA, has al-
ready been shown to be enhanced by IL-10 (49–51).

The host’s reactivity to LPS is a key part of a defensive 
immune/inflammatory response to gram-negative bacteria. 
Containment and elimination of infectious organisms are ac-
complished in part through the production of inflammatory medi-
ators, however, excessive production of these same mediators 
can result in pathology ranging from tissue damage to lethal 
endotoxic shock. Regulatory mechanisms have evolved to limit 
immune/inflammatory responses in order to avoid immunopath-
ologic damage. Our studies demonstrate that IL-10 has a central 
role in regulating the host’s response to endotoxin. The ability 
of IL-10 to suppress directly or indirectly the LPS-induced pro-
duction of pro-inflammatory mediators appears critical in de-
termining the host’s susceptibility to pathogenic reactions as no 
compensatory mechanisms existed in the IL10T mice which 
substituted for the IL-10 deficiency. Our studies also suggest 
that IL-10 may have a therapeutic role in the modification of 
responses to LPS.

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2346 Berg et al.


