IL-12, IL-18 and IFN-gamma in the immune response to bacterial infection

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Citation for published version (APA):
Lauw, F. N. (2000). IL-12, IL-18 and IFN-gamma in the immune response to bacterial infection.
Chapter 14

Epinephrine inhibits the production of interferon-\(\gamma\) and interleukin-12 in whole blood stimulated with endotoxin

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**Abstract**

Systemic inflammation leads to the activation of multiple host inflammatory responses, including the cytokine network and the release of stress hormones. To determine the effects of epinephrine on the release of IFN-γ and IL-12, two proinflammatory cytokines which have been implicated to contribute to the pathogenesis of sepsis and endotoxemia, human whole blood was stimulated in vitro with increasing concentrations of epinephrine. Epinephrine dose-dependently inhibited IFN-γ and IL-12p70 production stimulated by several bacterial stimuli, an effect which was mediated by an effect of epinephrine on β adrenergic receptors, since it could be prevented by addition of a specific β-adrenergic antagonist, while an α-adrenergic antagonist had no effect. In addition, incubation with the cAMP analog db-cAMP dose-dependently inhibited IFN-γ release stimulated by LPS. In the presence of anti-IL-12, epinephrine did not affect IFN-γ concentrations in LPS-stimulated whole blood, suggesting that epinephrine reduces IFN-γ release indirectly through inhibition of IL-12 production. These data suggest that, during acute infection, epinephrine may attenuate excessive release of IFN-γ and IL-12, but may also contribute to a decreased Th1 type immune response.
Introduction

Interferon-γ (IFN-γ) is an important proinflammatory cytokine which is mainly produced by CD4+ T helper 1 (Th1) cells, CD8+ T cells and natural killer (NK) cells (1). IFN-γ is a potent stimulator of monocyte/macrophage activities, including the production of cytokines and phagocytosis (1, 2). IFN-γ has been demonstrated to play an important role during sepsis and endotoxemia. Elevated plasma concentrations of IFN-γ can be found in a subset of patients with sepsis, and during experimental sepsis in primates (3-6). During experimental endotoxemia in mice, neutralization of IFN-γ protected against lethality, while IFN-γ receptor deficient mice were resistant against endotoxin (LPS)-induced shock (7, 8).

Interleukin-12 (IL-12) is a heterodimeric proinflammatory cytokine formed by a p35 and a p40 subunit, which is produced mainly by antigen-presenting cells. IL-12 plays a central role in both innate immunity by stimulation of T and NK cell activity, and adaptive immunity by promoting the differentiation of naive T cells into Th1 type cells (9, 10). Elevated levels of IL-12 have been measured in during clinical and experimental sepsis (4-6). IL-12 has been demonstrated to be important for IFN-γ production during endotoxemia in mice, and neutralization of IL-12 protected against LPS-induced lethality (11, 12).

Administration of LPS to healthy humans leads to the activation of the cytokine network and induces a characteristic stress hormone release (13). Several studies have demonstrated that stress hormones can influence the production of cytokines during experimental human endotoxemia. Infusion of epinephrine or glucocorticoids have been shown to alter the cytokine response elicited by LPS characterized by reduced TNF production and enhanced IL-10 release (14-16). Knowledge of the effects of epinephrine on IFN-γ and IL-12 release is limited. Such knowledge may contribute to the understanding of the endogenous effects of stress hormones during acute systemic infection on the cytokine network, and also for the therapeutic use of catecholamines in patients with systemic shock. It is difficult to study the effects of epinephrine on IFN-γ and IL-12 release induced by LPS in humans in vivo, since in the widely used model of human endotoxemia, IFN-γ and IL-12 concentrations are hardly detectable. Therefore, in the present study, we studied the effect of epinephrine on IFN-γ and IL-12 production by different bacterial stimuli during whole blood stimulation in vitro, a condition which is considered to mimic the human in vivo condition most closely (17). In addition, since IL-12 is known to be important stimulator of IFN-γ production, we examined whether epinephrine influences IFN-γ production directly or whether this effect is mediated through effects of epinephrine on IL-12 release.
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Materials and methods

Reagents
LPS (from *Escherichia coli* serotype 0111:B4) and Staphylococcal enterotoxin B (SEB) were obtained from Sigma (St. Louis, MO). Heat-killed *Staphylococcus aureus* (HKSa) were prepared from reference strain 14459B from the National Institute of Public Health and the Environment (Bilthoven, the Netherlands). The isolate was suspended in 50 ml Todd-Hewitt broth and cultured overnight in 5% CO2 at 37°C. This suspension was diluted in fresh medium the next morning and incubated until log-phase growth was obtained. Thereafter, 10-fold dilutions of this suspension were made and plated on blood agar plates for colony-forming unit (CFU) counts. Bacteria were harvested by centrifugation, washed twice in pyrogen-free 0.9% NaCl, resuspended in 20 ml 0.9% NaCl, and heat inactivated for 60 minutes at 80°C. A 500-μl sample on a blood agar plate did not show growth of bacteria. Dilutions were made in pyrogen-free RPMI 1640 (Bio Wittaker, Verviers, Belgium).

Adrenergic agents were obtained from the following manufacturers: epinephrine from Centrafarm Services (Etten-Leur, the Netherlands), phenolamine from Ciba-Geigy (Basel, Switzerland), and propranolol from Zeneca (Ridderkerk, the Netherlands). Dibutyryl-cyclic AMP (db-cAMP) was purchased from Sigma. Neutralizing mouse-anti-human IL-12 MAb and control mouse IgG were obtained from R&D Systems (Abingdon, UK).

Whole blood stimulation
Whole blood was collected aseptically from 6 healthy individuals using a sterile collecting system consisting of a butterfly needle connected to a syringe (Becton Dickinson & Co, Rutherford, NJ). Anticoagulation was obtained using endotoxin-free heparin (Leo Pharmaceutical Products B.V., Weesp, the Netherlands; final concentration 10 U/ml blood). Whole blood, diluted 1:1 in RPMI, was stimulated for 24 h at 37°C with LPS (10 ng/ml), HKSa (10^7 CFU/ml) or anti-CD/anti-CD28 double stimulation (both 1:1000), in the presence or absence of increasing concentrations of epinephrine (10^{-7}-10^{-5} M). To determine by which mechanism epinephrine exerts its effects, whole blood was stimulated with LPS (10 ng/ml) with or without epinephrine (10^{-6} M), phenolamine (10^{-5} M), propranolol (10^{-5} M), or db-cAMP (10^{-6}-10^{-4} M). In some experiments, neutralizing mAbs directed against human IL-12 or isotype-matched control mouse IgG were used (both 10 μg/ml). This concentration of anti-IL-12 completely neutralizes activity of recombinant human (rh)IL-12 when added at 1-2 log higher concentrations compared with levels found after whole blood stimulation with LPS (information on the neutralizing capacity of the mAb was provided by the manufacturer). After the incubation, supernatant was obtained after centrifugation (1600 x g for 15 minutes at 4°C) and stored at -20°C until assays were performed.
Epinephrine inhibits IFN-γ and IL-12 production

Assays

IFNγ and IL-12p70 were measured by specific ELISAs according to the instructions of the manufacturer (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, the Netherlands). The detection limits of the assays were 2.4 pg/ml (IFN-γ) and 6.2 pg/ml (IL-12p70).

Statistics

All data are expressed as mean ± SE of six donors. Statistical analysis was performed by Wilcoxon test. P < 0.05 was considered to represent a significant difference.

Results

Effect of epinephrine on IFN-γ and IL-12 production induced by different bacterial stimuli

Incubation of whole blood for 24 h at 37°C without stimulus did not result in detectable levels of IFN-γ or IL-12p70. Also, epinephrine did not induce cytokine production when added in the absence of a stimulus. Incubation with LPS, HKSa or SEB resulted in high concentrations of IFN-γ, while low concentrations of IL-12p70 were found (Figure 1). Addition of epinephrine induced a strong dose-dependent inhibition of IFN-γ and IL-12p70 after stimulation with LPS or HKSa, while the inhibitory effect on SEB cytokine production was less strong.

![Figure 1](image-url)

Figure 1. Epinephrine dose-dependently inhibits LPS-stimulated IFN-γ and IL-12p70 release in whole blood. Whole blood from six different donors, diluted 1:1 in RPMI, was incubated for 24 h at 37°C, with LPS (10 ng/ml), HKSa (10^7 CFU/ml) or SEB (1 µg/ml) in the presence or absence of increasing concentrations of epinephrine. Data are expressed as percentage change (mean ± SE) relative to incubation with LPS, HKSa or SEB only. IFN-γ concentrations were 10.01 ± 2.65 ng/ml (LPS only), 16.61 ± 7.2 ng/ml (HKSa), and 11.91 ± 4.68 ng/ml (SEB). IL-12p70 concentrations were 73.46 ± 37.11 pg/ml (LPS), 62.92 ± 23.86 pg/ml (HKSa), and 41.56 ± 26.29 pg/ml (SEB). * P < 0.05 vs. LPS, HKSa or SEB only.
Epinephrine inhibits IFN-γ production mainly through β-adrenergic stimulation

Since it is known that epinephrine can bind to both α (α₁ and α₂) and β (β₁ and β₂) adrenergic receptors, we sought to determine whether the effect of epinephrine on IFN-γ production was mediated through α or β adrenergic stimulation. Therefore, whole blood was incubated with LPS and epinephrine (10⁻⁶ M) in the presence or absence of a specific α adrenergic receptor antagonist phentolamine (10⁻⁵ M), and/or the specific β receptor antagonist propranolol (10⁻⁵ M). In the absence of epinephrine, neither phentolamine nor propranolol affected LPS-induced cytokine production. Epinephrine again strongly inhibited LPS-induced IFN-γ production (Figure 2). α receptor blockade with phentolamine had no effect on epinephrine-induced inhibition of LPS-induced IFN-γ release. In contrast, β receptor blockade with propranolol completely prevented the inhibiting effect of epinephrine. The combination of propranolol and phentolamine resulted in IFN-γ concentrations similar to those found after addition of propranolol alone. These data suggest that epinephrine inhibits LPS-induced IFN-γ production by an effect on the β-adrenergic receptor.

**Figure 2.** Effect of α and/or β adrenergic receptor blockade on IFN-γ production by LPS-stimulated whole blood. Whole blood from six different donors, diluted 1:1 in RPMI, was incubated for 24 h at 37°C, with LPS (10 ng/ml). Epi, with epinephrine (epi, 10⁻⁶ M). A, with epinephrine and the α antagonist phentolamine (10⁻⁵ M). B with epinephrine and the β antagonist propranolol (10⁻⁵ M). C, with epinephrine and phentolamine and propranolol. Data are expressed as percentage change (mean ± SE) relative to incubation with LPS only. * P < 0.05 vs. LPS only.

db-cAMP inhibits IFN-γ release

β-adrenergic stimulation is known to result in increased levels of intracellular cAMP (18). Therefore, we wanted to determine the effects of db-cAMP on LPS-induced IFN-γ production. The addition of dbcAMP caused a dose-dependent inhibition of IFN-γ release stimulated by LPS (Fig. 3).

Effect of anti-IL-12 on epinephrine-induced inhibition of IFN-γ production

It has been demonstrated in vitro and in mice that LPS-induced IFN-γ production is largely dependent on IL-12 (11, 12, 19). Having established that epinephrine inhibits IL-12p70 production stimulated by LPS, we were interested whether the inhibiting effect of
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Epinephrine on IL-12p70 production contributes to epinephrine-induced inhibition IFN-γ. Therefore, whole blood was incubated with LPS in the presence or absence of epinephrine (10⁻⁶ M), a neutralizing anti-IL-12 Ab, or an irrelevant control Ab (10 μg/ml). Anti-IL-12 strongly reduced LPS-induced IFN-γ (Table 1). In the presence of anti-IL-12, addition of epinephrine had no effect on LPS-induced IFN-γ release.

Table 1. Epinephrine does not affect LPS-induced IFN-γ production in the presence of anti-IL-12.

<table>
<thead>
<tr>
<th>Condition</th>
<th>IFN-γ (ng/ml)</th>
</tr>
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<tbody>
<tr>
<td>LPS</td>
<td>9.39 ± 3.06</td>
</tr>
<tr>
<td>LPS + epi</td>
<td>4.05 ± 2.19 *</td>
</tr>
<tr>
<td>LPS + anti-IL-12</td>
<td>1.30 ± 0.57 *</td>
</tr>
<tr>
<td>LPS + anti-IL-12 + epi</td>
<td>1.13 ± 0.58 **</td>
</tr>
</tbody>
</table>

Values are mean ± SE of six different donors. Whole blood, diluted 1:1 in RPMI, was incubated for 24 h at 37°C with LPS (10 ng/ml) in the presence or absence of epinephrine (epi, 10⁻⁶ M) and/or anti-IL-12 (10 μg/ml). * P < 0.05 vs. LPS only. ** P < 0.05 vs. LPS + epi.

Discussion

During acute infection, both the production of cytokines and the release of stress hormones are increased. Previous studies have established that complex interactions exist between stress hormones and the cytokine network. Administration of proinflammatory cytokines including TNF, IL-1 and IL-6 induce the release of stress hormones in humans and primates (20-22). Further, the catecholamines epinephrine and norepinephrine have been shown to inhibit the production of LPS-stimulated TNF, IL-6 and IL-1β in vitro, while increasing the release of IL-10 (15, 18, 23). Also, infusion of epinephrine or glucocorticoids in humans
inhibited TNF release during experimental endotoxemia, while enhancing IL-10 secretion (14-16).

We demonstrate here that epinephrine inhibits production of IFN-γ and IL-12 induced by several bacterial stimuli during whole blood stimulation in vitro. Similar concentrations of epinephrine have been used in previous in vitro studies to determine the effect of epinephrine on cytokine release (15, 23, 24). The inhibitory effects of epinephrine was more pronounced on LPS and HKSa-stimulated IFN-γ and IL-12 production compared to the release induced by SEB. This discrepancy may reflect the different mechanism of cell activation involved with the stimuli, i.e. LPS and HKSa mainly activate monocytes/macrophages, while the bacterial superantigen SEB, after simultaneously binding to MHC class II molecules of an APC and the specific Vβ domain of the T cell receptor, activates large fractions of the T cell population (25). Epinephrine inhibited IFN-γ release by an effect on β-adrenergic receptors. Indeed, β-adrenergic blockade by propranolol completely prevented the inhibition of epinephrine on LPS-induced IFN-γ production. In contrast, α-adrenergic blockade by phentolamine did not influence IFN-γ release.

Elevation of intracellular cAMP levels is a well-known postreceptor effect of β-adrenergic stimulation (18). Incubation with the cAMP analog db-cAMP dose-dependently inhibited IFN-γ release stimulated by LPS. In previous studies, prostaglandin-E2 (PGE2), an inflammatory mediator known to increase intracellular levels of cAMP, was reported to also inhibit LPS-induced IL-12 production, an effect which could be mimicked by other cAMP inducers (26, 27). Also, β2-agonists have been reported to inhibit IL-12 production from monocytes and dendritic cells, which was associated with increased concentrations of intracellular cAMP (28). Together, these data suggest that changes in intracellular concentrations of cAMP are involved in the suppression of IFN-γ and IL-12 production by epinephrine.

IL-12 is a important for the regulation of IFN-γ in vitro and in mice during endotoxemia (11, 12, 19) and the present study). Since epinephrine potently inhibited LPS-induced IL-12 production, the reduction of IFN-γ release could in part be the result of the inhibiting effect on IL-12. To eliminate the effect of reduced IL-12 concentrations, experiments with a neutralizing Ab against IL-12 were performed. In the presence of anti-IL-12, epinephrine did not affect IFN-γ concentrations in LPS-stimulated whole blood. This suggests that epinephrine reduces IFN-γ release during whole blood stimulation with LPS indirectly through inhibition of IL-12 production.

We used the whole blood assay to study the effects of epinephrine, rather than cultures of isolated cells. The use of whole blood eliminates possible artifacts that may result from isolation of cells, such as an alteration in cytokine-producing capacity (29). In addition, the effect of a hormone on cytokine release in whole blood can be studied under conditions
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with a physiological endocrine background and in the presence of all blood components, which is likely to be of more relevance for the in vivo situation.

Systemic inflammation leads to the activation of multiple host inflammatory responses, including the cytokine network and the release of stress hormones (13). We here report that epinephrine strongly inhibits LPS-stimulated IL-12 production and, indirectly of IFN-γ, in whole blood in vitro mainly through β-adrenergic stimulation. IL-12 plays an important role in the regulation of Th1/Th2 balance by promoting the differentiation of Th1 type cells (9, 10). A Th1-mediated immune response, accompanied by the production of Th1 type cytokines including IFN-γ, is associated with cell-mediated immunity (30). Therefore, release of epinephrine during the acute phase of inflammation attenuates excessive proinflammatory cytokine release, but may also contribute to a decreased Th1 type immune response, which may render the host more susceptible to concurrent infection.

References

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