IFN-γ is not induced through increased plasma concentrations of interleukin-12/interleukin-18 during human endotoxemia

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ABSTRACT. Endotoxin administration to animals and humans is an accepted experimental model of Gram-negative sepsis, and endotoxin is believed to play a major role in triggering the activation of cytokines. In septic patients, the IL-12/IL-18/IFN-γ axis is activated and correlates with mortality. Our aim was to investigate the effects of endotoxin administration in humans on the activation of the IL-12/IL-18/IFN-γ axis. Seven healthy volunteers received *E. coli* endotoxin (O:113). Hemodynamics, temperature and the course of plasma concentrations of TNF-α, IL-1β, IL-12, IL-18 and IFN-γ were determined. Endotoxin administration resulted in the expected flu-like symptoms, a temperature of 38.8 ± 0.3°C ($p < 0.003$), a decrease in mean arterial blood pressure of 14.8 ± 1.8 mmHg ($p < 0.0002$) and an increase in heart rate of 27.5 ± 4.8 bpm ($p < 0.002$) compared to baseline values. TNF-α increased from 16.6 ± 8.2 to 927 ± 187 pg/mL ($p < 0.003$). IL-1β increased from 8.6 ± 0.5 to 25.3 ± 2.0 pg/mL ($p < 0.0001$). IL-12 showed no significant increase (8.2 ± 0.2 to 9.3 ± 0.8 pg/mL, $p = 0.13$), and all IL-18 measurements remained below the level of detection. In contrast, IFN-γ showed an increase from 106.6 ± 57.1 to 152.7 ± 57.8 ($p < 0.005$). These results indicate that pathways other than the IL-12/IL-18 axis may induce IFN-γ production in human endotoxemia.

Keywords: endotoxemia, human, IFN-γ, IL-12, IL-18, lipopolysaccharide

Stimulation of the cytokine network is of pivotal importance in the development of sepsis. Endotoxin administration to animals and humans is an accepted experimental model of Gram-negative sepsis, and endotoxin is believed to play a major role in triggering the activation of cytokines.

Interleukins 12 and 18 (IL-12, IL-18) act synergistically in inducing interferon-γ (IFN-γ) production [1]. Tumour necrosis factor α (TNF-α) and IL-1β also have IFN-γ-stimulating properties, although to a lesser extent and only in the presence of IL-12 [2-4].

In patients with sepsis, IL-12/IL-18 and IFN-γ levels are elevated and correlate with mortality [5, 6]. Animal experiments have demonstrated that in response to *E. coli* endotoxin administration, the IL-12/IL-18/IFN-γ axis is activated and the level of activation correlates with mortality [7]. Moreover, IL-18 antibodies prevent the endotoxin-induced IFN-γ increase and mortality [8].

In human endotoxemia, the interplay between IL-12, IL-18 and IFN-γ has not been determined. In view of the putative key role of IL-12 and IL-18 in the induction of IFN-γ during inflammation, we investigated the effects of human endotoxemia on the IL-12/IL-18/IFN-γ axis.

METHODS

Patients

After approval from the local ethics committee, seven non-smoking, healthy volunteers gave their written informed consent to participate in this study. Screening of the subjects prior to the experiment revealed no abnormalities in medical history, physical examination or routine laboratory tests, and all subjects tested negative for HIV and Hepatitis B. Subjects who had suffered a febrile illness in the two weeks prior to the experiments or who were taking prescription drugs (except for oral contraceptives) were excluded.

Experimental design

At t = 0 hrs, 2 ng/kg endotoxin (*Escherichia coli* O:113, United States Pharmacopia Convention, Rockville, MD, USA), was injected intravenously. Heart rate was continuously monitored using a 3-lead ECG, starting approximately two hours before endotoxin administration and was continued until the end of the experiment. Blood pressure was also continuously determined using an arterial pressure monitoring line (Viggo Spectramed, 5269-129) and a

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20 gauge intra-arterial catheter (Angiomat, Deseret Medical, Becton Dickinson, Sandy UT, USA) in the radial artery. Temperature was measured with an infrared tympanic thermometer (Sherwood Medical, Hertogenbosch, the Netherlands) every 30 min throughout the experiment. Plasma concentrations of TNF-α, IL-1β, IL-12p70, and IFN-γ were measured using simultaneous Luminex Assays [9] (R&D Systems, Minneapolis MN, USA), whereas IL-18 concentrations were determined using a commercial ELISA (Bender Medical Systems, Vienna, Austria) according to the manufacturer's instructions. Plasma concentrations of these cytokines were measured twice prior to endotoxin administration (and averaged), at t = 15, 30, 60 and 90 minutes, and at t = 2, 3, 4, 6, 8, 12 and 22 hrs to determine the peak value of each cytokine per individual.

Calculations and statistical analysis

Data are expressed as mean ± SEM unless otherwise specified. The data expressed for heart rate and mean arterial pressure were averaged for the 30 minutes prior to endotoxin administration, and the 30 minute period when the endotoxin effects were most prominent.

To analyse the effect of endotoxin administration on cytokine concentrations, a paired Student's *t*-test was performed to compare the baseline and peak values. A p value of < 0.05 was considered to indicate significance.

RESULTS

Age, height, and weight of the four female and three male volunteers averaged (mean ± SD) 23.9 ± 3.1 yrs, 175 ± 14 cm and 69.9 ± 13.7 kg respectively. Endotoxin administration induced the expected flu-like symptoms after 1 to 2 hrs. Body temperature increased from 36.8 ± 0.2 °C at baseline to maximally 38.8 ± 0.3 °C ($p < 0.003$) at 3 hrs 43 min ± 18 min after the administration of endotoxin. Heart rate increased from 62.7 ± 2.8 to maximally 90.1 ± 3.9 bpm (t = 3 hrs, $p < 0.002$), mean arterial pressure decreased from 97.0 ± 3.8 to minimally 82.2 ± 4.5 mmHg (t = 5 hrs, $p < 0.0002$).

Figure 1 shows the endotoxin-induced changes in cytokine concentrations over time. *Table 1* illustrates the group average of the individual peak concentrations of the cytokines measured. TNF-α concentration markedly increased ($p < 0.003$) and reached its zenith at t = 99 ± 6 min. Also, concentrations of IL-1β showed a significant increase ($p < 0.0001$) that peaked at t = 2 hrs 51 min ± 20 min. In contrast, the concentration of IL-12 did not increase significantly ($p = 0.13$), and IL-18 levels remained below the detection limit, while the IFN-γ concentration increased significantly ($p < 0.005$) at t = 2 hrs 30 min ± 20 min.

DISCUSSION

Our study demonstrates that human endotoxemia results in a significant increase in IFN-γ in the absence of detectable IL-12 and IL-18 production, indicating that a pathway

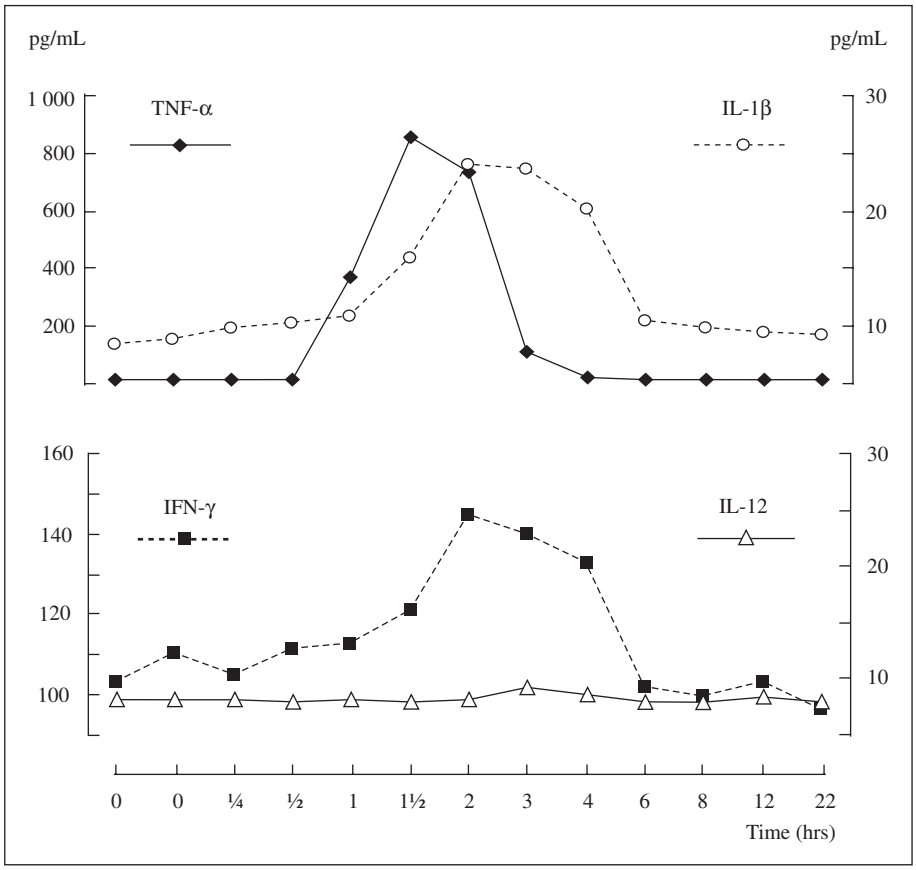

Figure 1

Time course of endotoxin-induced changes in cytokine concentrations.

Effect of 2 ng/kg *Escherichia coli* endotoxin administration to healthy volunteers on plasma concentrations of TNF-α, IL-1β, IFN,γ and IL-12. Endotoxin was administered after two baseline measurements at t = 0 hours. Data are expressed as mean values of seven subjects. For reasons of clarity SEMs are omitted. TNF-α, IL-1β, and IFN-γ showed significant increases (see also *table 1*), while there was no significant change in the IL-12 concentration.

Table 1
Cytokine levels in healthy volunteers before and after endotoxin administration

	Baseline concentration (pg/mL)	Peak concentration (pg/mL)	*p*-value
TNF-α	16.6 ± 8.2	927.0 ± 187.1	< 0.003
IL-1β	8.6 ± 0.5	25.3 ± 2.0	< 0.0001
IL-12	8.2 ± 0.2	9.3 ± 0.8	0.13
IL-18	Below detection limit	Below detection limit	
IFN-γ	106.6 ± 57.1	152.7 ± 57.8	< 0.005

Data are expressed as mean ± SEM. Baseline values are averaged for two measurements at t = 0 (before endotoxin). Peak concentration is the group average of the highest cytokine concentration of each subject. The p value refers to the statistical difference measured by paired Student's *t* test. The detection limit for IL-18 was 625 pg/mL.

other than the IL-12/IL-18 axis induces IFN-γ production in humans. This observation confirms previous human endotoxemia studies in which it was demonstrated that IL-18 was not induced during human endotoxemia [6, 10], but contrasts with animal experiments in which endotoxin was shown to increase IL-12 and IL-18 production [7]. Although the endotoxin dose used in animals is much higher, the very high TNF-α levels, the clinical response and the hemodynamic changes that we found, indicate that the inflammatory stimulus in human endotoxemia experiments is adequate.

IL-1β and TNF-α have also been identified as IFN-γ-inducing factors, and were both increased after the administration of endotoxin. However in animal experiments, in the absence of IL-12, neither IL-1β nor TNF-α were able to induce IFN-γ in endotoxemia [2-4]. It may be hypothesized that, in humans, IL-1β and TNF-α are able to induce IFN-γ in the absence of IL-12. To our knowledge, this potential pathway of IFN-γ induction in human endotoxemia has not been clarified and remains to be elucidated. *In vivo* experiments [10] have demonstrated that administration of a high dose of rhIL-10 before endotoxin, completely prevents the induction of the IL-12 subunit p40. This subunit appears to be crucial for IFN-γ induction [11]. In these experiments, IL-18 remained unaltered by both the endotoxin and the rhIL-10. However, the rhIL-10 enhanced IFN-γ concentrations, indicating that IFN-γ was also not induced through the IL-12/IL-18 axis.

Since elevated concentrations of IL-18 are found in septic patients [5, 6], it could also be argued that endotoxin administration to volunteers is not an appropriate model to study the activation of the cytokine network. Interestingly, in human sepsis, infections with Gram positive microorganisms demonstrated a much higher increase in IL-18 than that found in Gram negative sepsis [5]. Moreover, *in vitro* experiments [12] have demonstrated that Gram negative *C. pneumonia* is a much more potent inducer of IL-18 than LPS, indicating that other bacterial components apart from LPS may be the main driving force for activation of the IL-12/IL-18/IFN-γ axis. Future experiments using other bacterial components or the Gram positive equivalent of LPS, lipoteichoic acid (LTA) could help to clarify the interplay between IL-12, IL-18 and IFN-γ during Gram positive infections.

Lastly, it is well known that other proinflammatory cytokines are mainly produced by tissue macrophages in various organs, and not in the circulation [13]. A similar situation could be envisaged for IL-18, resulting in activation of the IL-12/IL-18/IFN-γ axis in these organs.

However, it remains unclear if this hypothesized IFN-γ induction would lead to increased plasma concentrations of IFN-γ. *In vitro* experiments [14] have demonstrated that the stimulation of human peripheral blood mononuclear cells by LPS induces an increase in TNF-α, IL-1β and IFN-γ, while IL-18 concentrations remain low. However, when the IL-18 antagonist IL-18BP is added, IFN-γ induction is markedly decreased, indicating a role for IL-18 in IFN-γ induction while IL-18 concentrations remain low.

In conclusion, our data demonstrate that in human endotoxemia, induction of IFN-γ is independent of the induction of IL-12 and IL-18 in plasma. This indicates that the production of IFN-γ is mediated through an alternative pathway that remains to be identified.

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