Diurnal Variations in the Human Host Response to Endotoxin

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To investigate diurnal variations in the host response to endotoxin, Salmonella abortus equi endotoxin (0.8 ng/kg) was given intravenously to healthy men in a placebo-controlled design at 0900 or 1900 h. The time courses of rectal temperature and the plasma levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), adrenocorticotropic hormone (ACTH), and cortisol were monitored for 11 h following the injections. The time of day did not affect the endotoxin-induced increase in plasma TNF-α or IL-6. However, subjects who received endotoxin in the evening, when endogenous glucocorticoid levels were low, showed about twice the increases in rectal temperature and plasma ACTH and cortisol levels as those who received endotoxin in the morning, when endogenous glucocorticoid levels were high. These results demonstrate diurnal variations in the human susceptibility to endotoxin that may be due to a suppression of the biologic effects of TNF-α and IL-6 by endogenous glucocorticoids.

Circadian rhythms of many physiologic variables have been described [1, 2], including body temperature, endogenous glucocorticoid levels, and aspects of immune functions such as peripheral blood cell counts and in vitro responses of immunocompetent cells to stimulation in animals and humans [3]. In addition, toxicity of many cytotoxins can be reduced by appropriate circadian timing [4, 5]. However, little is known about diurnal variations in the human host response in vivo.

Endotoxin is a cell-wall lipopolysaccharide of gram-negative bacteria that is critically involved in the primary host response to infection [6, 7]. Endotoxin stimulates monocytes and macrophages to release cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α), which act in concert to induce a multitude of host responses, including fever [8] and activation of the hypothalamic-pituitary-adrenocortical (HPA) system [9]. Excessive endotoxin-induced cytokine release is thought to be involved in the development of gram-negative bacterial sepsis, which remains a major challenge to medical research because of its high incidence and 20%-80% lethality rate [10].

In mice, susceptibility to endotoxin is lowest in the middle of the active period, ~2400 h, and highest at the end of the resting period, ~1800 h [11]. The causes of these time-of-day differences are unknown. A recent study suggests that circadian variations in the toxicity of TNF-α may be involved. Hrushesky et al. [12] reported that TNF-α–induced lethality in mice varies up to 9-fold across the day, being lowest in the second half of the active period and highest at the end of the resting period. The only study published so far on the effects of endotoxin administration at various times of day in humans showed the highest endotoxin-induced increases in plasma adrenocorticotropic hormone (ACTH) and cortisol at 2300 h and the lowest at 0900 h [13]. In contrast, temperature response to endotoxin did not show diurnal variations. However, interpretation of these results is difficult because all volunteers received endotoxin twice within a few days, and repetitive administration is known to attenuate the host response [14].

To evaluate diurnal variations in the endotoxin-induced host response in more detail, we administered 0.8 ng/kg Salmonella abortus equi endotoxin intravenously to 2 groups of 10 healthy volunteers each, in a single-blind placebo-controlled experiment, either at 0900 or 1900 h. We monitored rectal temperature and plasma levels of IL-6, TNF-α, ACTH, and cortisol in both groups.

Materials and Methods

Salmonella abortus equi endotoxin. The isolation and purification of Salmonella abortus equi endotoxin has been described in detail [15–18]. The resulting preparation was free of protein (<0.08%) and nucleic acids. It was available as a sterile solution in ampules of 100 ng/mL endotoxin.

Subjects. Twenty healthy paid male volunteers were screened by medical history, physical examination, laboratory investigations, electrocardiography, and electroencephalography, to exclude current and chronic disease.

Experimental design. In all subjects, the effects of 0.8 ng/kg endotoxin and of 0.9% saline were evaluated in a single-blind randomized crossover design 1–2 weeks apart. On both occasions,
10 subjects received endotoxin or placebo at 0900 h; the 10 remaining volunteers were injected at 1900 h.

An intravenous catheter for intermittent blood sampling was placed before administration of endotoxin or placebo, and subjects remained in bed for 11 h after injection. They were under continuous observation, and an experienced physician was permanently on call. Blood samples were stabilized with Na-EDTA (for cytokine and cortisol assays) or 80°C (for ACTH assay) immediately and aliquoted and frozen at 4°C and 2600 g. Plasma was aliquoted and frozen to –20°C for cytokine and cortisol assays or –80°C (for determination of ACTH levels). Rectal temperature was assessed by a thermistor probe, and a one-lead electrocardiogram was monitored continuously.

Cytokine, ACTH, and cortisol assays. TNF-α and IL-6 were measured by ELISAs (Medgenix, Brussels). For both assays, the limit of detection was 3 pg/mL. Intra- and interassay coefficients of variation were <5% and <8%, respectively. TNF-α and IL-6 levels were not determined after placebo administration because they have been reported to be near the detection limit of the assays in unstimulated healthy subjects [19, 20]. ACTH and cortisol levels were assessed by use of coated tube RIA (Nichols Institute, San Juan Capistrano, and ICN Biomedicals, Carson, CA, respectively). The sensitivity of the cortisol assay was 1.5 ng/mL, and the limit of detection of the ACTH assay was 3 pg/mL. Intra- and interassay coefficients of variation were <10% for both assays.

Statistical methods. The time course of host response was compared between groups by repeated-measures analysis of variance (ANOVA). Post hoc comparisons were done with Student’s t tests for independent samples. For intragroup comparisons, the paired Student’s t test was used. Two-tailed P values are reported, and P < .05 was considered significant. In the text and tables, means ± SDs are given; in the figures, means ± SEs are depicted.

Results

Table 1 gives the baseline characteristics of the 2 groups of volunteers. There were no significant differences in age, height, weight, or preinjection plasma TNF-α and IL-6 levels. As expected, subjects who received endotoxin in the morning had significantly lower baseline rectal temperatures and heart rates and higher plasma ACTH and cortisol levels than volunteers given endotoxin in the evening. Because of these circadian baseline differences, the net effect of endotoxin administration on temperature, heart rate, and plasma ACTH and cortisol levels was determined by computing the difference between the endotoxin and placebo results for each subject at each time point.

Following administration of the endotoxin, the occurrences of symptoms such as muscle aches (3 vs. 3 subjects), short-lasting chills (5 vs. 3 subjects), headache (5 vs. 3 subjects), and sleepiness (9 vs. 4 subjects) were similar in the evening and morning treatment groups, respectively. Figure 1 shows the time courses of host response to endotoxin for both groups.

ANOVA for repeated measures did not yield significant time-condition interaction effects or significant condition effects for TNF-α or IL-6 levels or heart rate. Significant time-condition interaction effects were found for rectal temperature (F[10, 180] = 5.31, P < .001) and ACTH (F[10, 180] = 4.76, P < .001) and cortisol (F[10, 180] = 3.80, P < .001) plasma levels. In the evening treatment group, the endotoxin-evoked increases were greater for ACTH (4 and 6 h after injection) and cortisol (4–7 h after injection). However, following endotoxin-induced HPA system activation, ACTH (10 and 11 h after injection) and cortisol (9–11 h after injection) plasma levels were suppressed in the evening treatment group.

In line with this biphasic HPA system response to endotoxin in the evening treatment group, there was no significant condition effect for the ACTH (F[1, 18] = 0.24, P = 0.628) and cortisol (F[1, 18] = 0.13, P = 0.724) responses. The temperature response to endotoxin was overall greater in the evening treatment group, as reflected by a significant treatment effect (F[1, 18] = 5.69, P < .05). The temperature increase was significantly higher from 4 to 7 h after injection.

The interaction of endotoxin-evoked responses with the physiologic diurnal variations is illustrated by figure 2, which

<table>
<thead>
<tr>
<th>Morning treatment group (n = 10)</th>
<th>Evening treatment group (n = 10)</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) 25.7 ± 2.5</td>
<td>28.1 ± 6.8</td>
<td>1.03</td>
<td>.315</td>
</tr>
<tr>
<td>Height (cm) 184 ± 5</td>
<td>180 ± 7</td>
<td>1.35</td>
<td>.195</td>
</tr>
<tr>
<td>Weight (kg) 75.2 ± 5.9</td>
<td>72.4 ± 4.7</td>
<td>1.18</td>
<td>.252</td>
</tr>
<tr>
<td>Rectal temperature (°C) 36.5 ± 0.2</td>
<td>37.0 ± 0.2</td>
<td>4.64</td>
<td>.000</td>
</tr>
<tr>
<td>Heart rate (bpm*) 58 ± 10</td>
<td>66 ± 9</td>
<td>2.11</td>
<td>.049</td>
</tr>
<tr>
<td>Plasma TNF-α (pg/mL) 3.5 ± 3.6</td>
<td>1.8 ± 3.6</td>
<td>1.60</td>
<td>.333</td>
</tr>
<tr>
<td>Plasma IL-6 (pg/mL) 7.6 ± 17.5</td>
<td>0.0 ± 0.1</td>
<td>1.37</td>
<td>.189</td>
</tr>
<tr>
<td>Plasma ACTH (pg/mL) 26.9 ± 5.6</td>
<td>18.4 ± 8.1</td>
<td>2.64</td>
<td>.017</td>
</tr>
<tr>
<td>Plasma cortisol (ng/mL) 134.8 ± 53.3</td>
<td>51.8 ± 23.1</td>
<td>4.52</td>
<td>.000</td>
</tr>
</tbody>
</table>

NOTE. bpm, beats/min; TNF, tumor necrosis factor; IL, interleukin; ACTH, adrenocorticotropic hormone.
Activation of the host defense by endotoxin involves a cascade of events starting with the binding of endotoxin to endotoxin-binding protein. This complex activates immunocompetent cells, mainly monocytes and macrophages, through the CD14 receptor [21]. These cells then release mediators, such as the cytokines IL-1β, TNF-α, and IL-6 (reviewed in [6]).

During experimental low-dose endotoxemia in humans, TNF-α and IL-6 are usually detectable in the circulation, whereas IL-1β generally is not [7, 20, 22-24]. Furthermore, blocking the biologic activity of IL-1β with IL-1 receptor antagonist in healthy volunteers fails to significantly attenuate the host response to endotoxin in humans [25, 26]. Therefore, TNF-α and IL-6, both known to increase temperature and plasma cortisol levels in humans [27 - 29], are likely to be important mediators of fever and activation of the HPA system during the physiologic plateau phase, and the early morning increase was attenuated.

Figure 1. Host response to endotoxin (0.8 ng/kg) in healthy volunteers injected at 0900 h (n = 10) or 1900 h (n = 10). Absolute plasma levels of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) following endotoxin injection are given. Due to large physiologic diurnal variations, rectal temperature, adrenocorticotropic hormone (ACTH), cortisol, and heart rate responses are depicted as differences between endotoxin and placebo sessions. Bars = SE. * P < .05 and ** P < .01 between groups.

Discussion

Activation of the host defense by endotoxin involves a cascade of events starting with the binding of endotoxin to endotoxin-binding protein. This complex activates immunocompetent cells, mainly monocytes and macrophages, through the CD14 receptor [21]. These cells then release mediators, such as the cytokines IL-1β, TNF-α, and IL-6 (reviewed in [6]). During experimental low-dose endotoxemia in humans, TNF-α and IL-6 are usually detectable in the circulation, whereas IL-1β generally is not [7, 20, 22-24]. Furthermore, blocking the biologic activity of IL-1β with IL-1 receptor antagonist in healthy volunteers fails to significantly attenuate the host response to endotoxin in humans [25, 26]. Therefore, TNF-α and IL-6, both known to increase temperature and plasma cortisol levels in humans [27 - 29], are likely to be important mediators of fever and activation of the HPA system in experimental low-dose endotoxemia, whereas a pivotal role of IL-1β is questionable.

In the present investigation, endotoxin-induced increases in plasma TNF-α and IL-6 levels showed no diurnal variation, whereas temperature, ACTH, and cortisol responses to endotoxin were considerably greater after administration at 1900 h than at 0900 h. These results suggest that, in humans, it is not the endotoxin-induced release of TNF-α and IL-6 that varies
Figure 2. Rectal temperature and plasma adrenocorticotropic hormone (ACTH) and cortisol levels during placebo and endotoxin conditions on local time scale. Note that 2 sets of experiments in different groups of volunteers are depicted (n = 10 for each group). Bars = SE. * P < .05 and ** P < .01 between placebo and endotoxin conditions, respectively.

during the day but rather the sensitivity of the host defense system to the actions of these cytokines. This idea is in line with the recently reported diurnal variations in TNF-α–induced lethality in mice [12]. Elliot et al. [30] reported a similar time-of-day variability in endotoxin-induced lethality in mice that was paralleled by variations in serum TNF levels. Because a bioassay based on lysis of murine L929 cells was used, it cannot be distinguished whether these results indicate a diurnal variation in endotoxin-induced TNF-α protein release or TNF-α bioactivity (or both).

Diurnal variations in the effects of TNF-α and IL-6 on rectal temperature and the HPA system may be related to the circadian rhythm in plasma cortisol levels, because endogenous glucocorticoids are potent modulators of cytokine actions [31]. For example, adrenalectomy [32] and glucocorticoid receptor type II blockade [33] both sensitize animals to the lethal effects
of TNF-α, suggesting that endogenous glucocorticoids play a protective role against the toxic effects of cytokines. This view is further supported by studies demonstrating that glucocorticoids reduce TNF receptor affinity [34] and inhibit the cytotoxic activity of TNF on murine L929 cells [35]. These suppressive effects of glucocorticoids on cytokine actions may explain our finding that, despite comparable TNF-α and IL-6 responses, endotoxin-induced temperature increase and HPA system activation were less pronounced in the morning, when preinjection plasma cortisol levels were higher than in the evening. Pharmacologic doses of glucocorticoids suppress endotoxin-induced TNF-α release [36–39]. Our results suggest that this is not the case for endogenous glucocorticoids at concentrations in the range of their physiologic diurnal variation.

Prostaglandins have been suggested to play a role in the generation of the circadian changes in body temperature, because prostaglandin synthesis inhibitors suppress the physiologic nocturnal temperature rise in rats [40]. In addition, prostaglandins are involved in both the pyrogenic and HPA system responses to endotoxin in humans, because ibuprofen blocks both temperature and cortisol increases [41–43]. This blunting of responses to endotoxin by ibuprofen cannot be explained by reduced endotoxin-induced increases in systemic TNF-α and IL-6 levels, because these are actually augmented [24]. Studies in rats suggest that HPA-system activation by TNF-α is mediated by prostaglandins in a manner independent of endogenous glucocorticoid feedback, because even in adrenal-ectomized animals, TNF-α-induced ACTH release can be blocked by indomethacin [44]. Therefore, diurnal variations in cytokine-induced prostaglandin synthesis may represent a corticosteroid-independent mechanism modulating the human host response to endotoxin in a 24-h period.

Our results clearly demonstrate a considerable diurnal variation in the effects of endotoxin in humans. Studies using more time points during the 24-h period should further elucidate the circadian variations in host response, thus allowing the exact definitions of the peak, nadir, and amplitude of the rhythm of endotoxin effects. Due to the complex interaction between physiologic diurnal variations in body temperature and plasma levels of ACTH and cortisol (figure 2), such studies should be placebo-controlled. To further clarify the role of endogenous glucocorticoids in the circadian modulation of host response to endotoxin, studies involving glucocorticoid type II receptor blockade would be helpful. However, such studies should be done in animals, because the expected increase in host response could be harmful for humans.

Appropriate timing of administration reduces the toxicity of many cytotoxins [4]. Furthermore, delayed hypersensitivity reaction [45], immediate cutaneous responses to intradermally injected allergens [46, 47], frequency and severity of asthma attacks [48], antibody response to vaccination [49, 50], and rejection of renal transplants [51, 52] all show diurnal variations. Therefore, detailed knowledge about the circadian aspects of the host defense system may be of considerable importance for the timing of many therapeutic interventions. In addition, this knowledge may enhance our understanding of the circadian pathophysiology of various diseases.

References


