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**Research article**

**Interleukin-18 does not modulate the acute-phase response**

Rogier J.L. Stuyt1,2, Mihai G. Netea1,2, Ineke Verschueren1,2, Charles A. Dinarello3, Bart Jan Kullberg1,2, Jos W.M. van der Meer1,2

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IL-18 is a pro-inflammatory cytokine of the IL-1 family and it induces IL-1, TNF, and IL-6, all of which are endogenous pyrogens. The pyrogenic properties of recombinant IL-18 were studied in a rabbit model of fever. rIL-18 did not cause fever when injected intravenously into rabbits. Furthermore, the ability of rIL-18 to modulate other components of the acute-phase response was assessed. rIL-18 did not induce leukocytosis, or changes of circulating concentrations of lipoproteins and corticosterone in mice. In conclusion, rIL-18 is not able to induce a febrile response in rabbits and does not modulate the acute-phase response in mice.

**Keywords:** Acute-phase response, fever, IL-18, IL-1

**INTRODUCTION**

IL-18, a cytokine of the IL-1 family, plays an important role in the Th1-type cytokine response, primarily by its ability to induce IFN-γ production by T cells and natural killer cells in synergy with IL-12 and microbial products.1,2 In addition, IL-18 induces IL-1, TNF, and IL-6, all well-established endogenous pyrogens.3,4 IL-18 and IL-1β share a series of characteristics, which is not surprising considering that IL-18 is structurally related to IL-1β.5,6 Both cytokines require caspase-1 (also known as IL-1β converting enzyme) for cleavage and release of their active mature form into the extracellular compartment. The ligand-binding IL-18 receptor (IL-18R) α-chain is similar to the IL-1R type I, and the signaling IL-18Rβ chain is similar to the IL-1R accessory protein. Furthermore, IL-18 and IL-1β signal transduction involve activation of identical cytoplasmic messengers – MyD88, IL-1 receptor-associated kinase, and tumor necrosis factor (TNF) receptor-associated factor-6. IL-1β is probably the most potent inducer of the acute-phase response.7,8 It is tempting to speculate that IL-18, which has pro-inflammatory properties, could also function as an inducer of the acute-phase response.

The endogenous pyrogens IL-1α, IL-1β, TNF-α, and IL-6 induce synthesis of prostaglandin E2 (PGE2), in the thermoregulatory center of the hypothalamus, which in turn elevates the hypothalamic temperature setpoint, leading to fever by efferent mechanisms.9,10 We have studied the pyrogenic properties of recombinant human IL-18 (rIL-18) in a rabbit model of fever. Moreover, the ability of rIL-18 to modulate other components of the acute-phase response such as leukocytosis, lipid profiles and glucocorticosteroid production was assessed.

**MATERIALS AND METHODS**

**Rabbit fever model**

New-Zealand rabbits (3.0–3.5 kg) were obtained from a local colony. Seven days before being used in the experiments, the rabbits were accustomed to the stocks. During the experiment, the animals were kept at 21 ± 1°C ambient temperature and housed with a 12-h light-dark
cycle. They had free access to food and water. Three New-Zealand rabbits per group were intravenously (i.v.) injected with LPS (50 ng/kg) or recombinant human IL-18 (1 µg/kg) according to Dinarello et al.11 and the temperature was monitored continuously for 5 h using a rectal thermometer.

Materials

Recombinant human and murine IL-18 was kindly provided by Dr M. Kurimoto (Fujisaki Institute, Hayashibara Biochemical Laboratories, Okayama, Japan). LPS (Escherichia coli O55:B5) was obtained from Sigma Chemical Co (St Louis, MO, USA).

Induction of the acute-phase response in mice

C57Bl/6J mice (females, 20–25 g, 6–8 weeks old) were obtained from Laboratory (Bar Harbour, ME, USA). Animals were housed under standard laboratory conditions and fed sterilized laboratory chow (Hope Farms, Woerden, The Netherlands) and water ad libitum. Mice were injected intravenously with rmIL-18 (1 µg/mouse) in 100 µl of sterile pyrogen-free PBS. Control mice received 100 µl of sterile pyrogen-free PBS i.v. At various time-points, subgroups of four animals were killed, and blood was collected on EDTA for white blood cell counts, and measurements of cholesterol, triglycerides and corticosterone.

Total white blood cells were counted in a Coulter counter (Coulter Electronics, Mijdrecht, The Netherlands). Tail blood of mice was used to prepare thin blood films, which were stained with May–Grunwald Giemsa’s solutions. Differential counts of blood cells were performed by counting 200 nucleated cells from randomly selected fields.

Cholesterol and triglycerides were determined by enzymatic methods on a Hitachi 747 analyser. Corticosterone concentrations in plasma were determined by a specific radioimmunoassay as described by Sweep et al.12

Statistical analysis

The differences between groups were analyzed by Mann-Whitney U-test. Data are expressed as mean ± SD.

RESULTS

Effect of IL-18 and LPS on core body temperature of New-Zealand rabbits

As shown in Figure 1, LPS produced a biphasic rise in body temperature with a maximum of 1.2°C after 80 min and of 1.0°C after 3 h. The temperature returned to normal by 4 h. In contrast, intravenous injection of rhIL-18 did not induce any significant change in the body temperature.
**Effect of IL-18 on leukocyte counts, cholesterol, triglycerides, and corticosterone in mice**

White blood cell counts and serum concentrations of cholesterol, triglycerides, and corticosterone were used as markers of the acute-phase response after IL-18 injection. At various time points, mice were killed and total white blood cell counts and acute-phase proteins were measured. As shown in Table 1, most of these parameters were not influenced by injection of rmIL-18, with the exception of the white blood cell counts, which were lower compared to control 1 and 6 h after injection of rmIL-18 (1 h, 7.8 ± 1.9 versus 5.2 ± 1.1 x 10⁶ cells/ml; 6 h, 8.2 ± 0.6 versus 4.6 ± 0.9 x 10⁶ cells/ml, both P < 0.05). No differences were observed in the percentages of neutrophils, lymphocytes or monocytes. Corticosterone levels were higher 3 h after injection of rmIL-18 (543 ± 54 versus 915 ± 223 nmol/l, P < 0.05). No significant differences in serum concentrations of cholesterol and triglycerides were observed between mice injected with PBS or rmIL-18. Circulating concentrations of IL-6 were under the detection limit in both groups, despite IL-6 induction in vitro in peritoneal macrophages (data not shown).

**DISCUSSION**

We investigated the effect of IL-18 on the acute-phase response. Injection of recombinant IL-18 did not induce any of the components of the acute-phase response studied: rIL-18 did not cause fever when injected intravenously into rabbits; rIL-18 did not induce leukocytosis, modification of circulating concentrations of lipoproteins, or corticosterone in mice. Thus, IL-18 is not an endogenous pyrogen, and this notion is in agreement with previous studies, showing no changes in temperature in animals treated with intraperitoneal IL-18,

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>rIL-18</th>
<th>Control</th>
<th>rIL-18</th>
<th>Control</th>
<th>rIL-18</th>
<th>Control</th>
<th>rIL-18</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>White blood cell count (x 10⁶ cells/ml)</td>
<td>Cholesterol (mmol/l)</td>
<td>Triglycerides (mmol/l)</td>
<td>Corticosterone (nmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>7.6 ± 1.4</td>
<td>1.7 ± 0.4</td>
<td>0.74 ± 0.07</td>
<td>408 ± 75</td>
<td></td>
<td></td>
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<tr>
<td>30 min</td>
<td>7.0 ± 2.4</td>
<td>1.5 ± 0.3</td>
<td>0.85 ± 0.13</td>
<td>895 ± 49</td>
<td></td>
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</tr>
<tr>
<td>1 h</td>
<td>7.8 ± 1.9</td>
<td>1.7 ± 0.1</td>
<td>0.90 ± 0.13</td>
<td>795 ± 67</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>2 h</td>
<td>5.4 ± 0.4</td>
<td>1.8 ± 0.2</td>
<td>0.69 ± 0.11</td>
<td>930 ± 91</td>
<td></td>
<td></td>
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<tr>
<td>3 h</td>
<td>4.2 ± 1.7</td>
<td>1.7 ± 0.1</td>
<td>0.59 ± 0.03</td>
<td>543 ± 54</td>
<td></td>
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<tr>
<td>6 h</td>
<td>8.2 ± 0.6</td>
<td>1.8 ± 0.2</td>
<td>0.52 ± 0.12</td>
<td>563 ± 204</td>
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</tr>
<tr>
<td>24 h</td>
<td>9.0 ± 1.8</td>
<td>1.7 ± 0.4</td>
<td>1.09 ± 0.48</td>
<td>385 ± 176</td>
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</tr>
</tbody>
</table>

Mice were injected intravenously with recombinant murine IL-18 (1 µg/mouse). After different time points, mice were killed and blood was collected. Data represent the mean ± SD of 4 mice. 

*P < 0.05 versus control; bP < 0.05 versus time 0.
lipoprotein concentrations after administration. In addition, only a minor effect on corticosterone levels was observed 3 h after injection of IL-18.

CONCLUSIONS
Data presented here show that rIL-18 has a very limited role on the acute-phase response: it is not able to induce a febrile response in rabbits, has no effects on leukocytosis and lipoproteins, and modulates steroid concentrations only marginally during the acute-phase.

ACKNOWLEDGEMENT
We thank Rob van den Berg for the corticosterone measurements.

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