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Rogier J. L. Stuyt, Mihai G. Netea, Ineke Verschueren, Charles A. Dinarello ...+2 more authors

Institutions: Radboud University Nijmegen, Anschutz Medical Campus

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Research article

Interleukin-18 does not modulate the acute-phase response

Rogier J.L. Stuyt[1,2], Mihai G. Netea[1,2], Ineke Verschueren[1,2], Charles A. Dinarello[3], Bart Jan Kullberg[1,2], Jos W.M. van der Meer[1,2]

[1]*Department of Medicine, Radboud University Nijmegen Medical Center, and*
[2]*Nijmegen University Center for Infectious Diseases, Nijmegen, The Netherlands*
[3]*Department of Medicine, University of Colorado Health Sciences Center, Denver, Colorado, USA*

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 E_2 (PGE_2), 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

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Correspondence to: Mihai G. Netea MD, Department of Medicine (541), Radboud University Nijmegen Medical Center, PO Box 9101, 6500 HB Nijmegen, The Netherlands
Tel: +31 24 3618819; Fax: +31 24 3541734; E-mail: M.Netea@aig.umcn.nl

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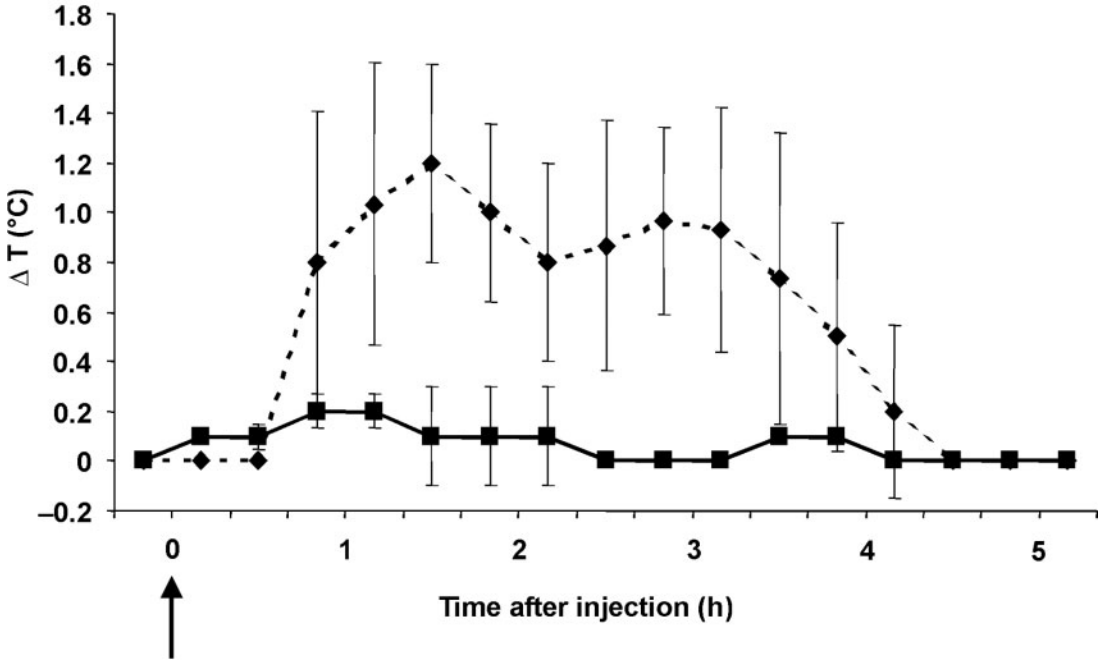

Fig. 1. Effect of IL-18 on the febrile response in rabbits. LPS (50 ng/kg) or recombinant human IL-18 (1 µg/kg) was injected intravenously into rabbits (n = 3 per group). The body temperature (in °C measured by rectal thermometer) was measured continuously for 5 h. The arrow indicates the timepoint of the injection of LPS or IL-18.

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.

Table 1. Effect of recombinant murine IL-18 on leukocyte counts, cholesterol, triglycerides, and corticosterone in mice at different timepoints

	White blood cell count (x 10^6 cells/ml)		Cholesterol (mmol/l)		Triglycerides (mmol/l)		Corticosterone (nmol/l)	
Time	Control	rIL-18	Control	rIL-18	Control	rIL-18	Control	rIL-18
0	7.6 ± 1.4	7.6 ± 1.4	1.7 ± 0.4	1.7 ± 0.4	0.74 ± 0.07	0.74 ± 0.07	408 ± 75	408 ± 75
30 min	7.0 ± 2.4	7.7 ± 3.3	1.5 ± 0.3	1.7 ± 0.2	0.85 ± 0.13	0.76 ± 0.03	895 ± 49[b]	810 ± 76[b]
1 h	7.8 ± 1.9	5.2 ± 1.1[a]	1.7 ± 0.1	1.7 ± 0.2	0.90 ± 0.13	0.94 ± 0.24	795 ± 67[b]	830 ± 73[b]
2 h	5.4 ± 0.4	7.6 ± 0.7	1.7 ± 0.1	1.6 ± 0.1	0.69 ± 0.11	0.65 ± 0.10	930 ± 91[b]	858 ± 72[b]
3 h	4.2 ± 1.7[b]	4.2 ± 0.6[b]	1.7 ± 0.1	1.7 ± 0.3	0.59 ± 0.03	0.69 ± 0.10	543 ± 54[b]	915 ± 223[a,b]
6 h	8.2 ± 0.6	4.6 ± 0.9[a,b]	1.8 ± 0.1	1.8 ± 0.2	0.52 ± 0.12	0.56 ± 0.10	563 ± 204	750 ± 87[b]
24 h	9.0 ± 1.8	8.7 ± 3.5	1.7 ± 0.4	1.9 ± 0	1.09 ± 0.48	0.78 ± 0.12	385 ± 176	238 ± 93[b]

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.
[a]$P < 0.05$ versus control; [b]$P < 0.05$ versus time 0.

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 is 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 10^6 cells/ml: 6 h, 8.2 ± 0.6 versus 4.6 ± 0.9 x 10^6 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 intraperitonial IL-18,[13–15] despite the observation that intracerebroventricular administration of IL-18 does increase brain temperature.[14]

The lack of pyrogenic effects of IL-18 may be due to the fact that despite the many similarities between IL-18 and IL-1β, there are also fundamental differences between these two cytokines. In contrast to IL-1β, which is not expressed constitutively and is strongly induced by bacterial stimuli, constitutive levels of IL-18 mRNA and protein are present in unstimulated cells and in organs of untreated, healthy animals.[16] These levels become only marginally increased by exogenous stimulation. The lack of response at the level of hypothalamus to IL-18 may also be related to the constitutive expression of IL-18 in the hypothalamus of rats with a normal temperature.[17] In the pathogenesis of fever, a central step is represented by the induction of prostaglandins by pyrogenic cytokines at the level of hypothalamus. Prostaglandins are considered the central mediators of fever. In line with the absence of pyrogenic activity, it has been recently shown that unlike IL-1β, IL-18 does not induce COX-2 expression in human mononuclear cells.[18] Moreover, IL-18 was able to suppress the induction of PGE_2 production by IL-1 and this suppressive effect was partly mediated by IFN-γ.[18] The absence of COX-2 and the lack of a pyrogenic effect of IL-18 may be explained by a recent study by Lee *et al.*[19] showing that IL-18 signal transduction in epithelial cells is primarily via the MAPK p38 pathway rather than NF-κB.

The other aspects of the acute-phase response studied (leukocytosis, lipoprotein concentrations, and steroid levels) were not, or only marginally, influenced by injection of rIL-18 in mice. IL-1 has multiple biological effects such as leukocytosis, increase in lipoprotein concentrations and corticosterone levels.[8,20] Despite the many similarities between the IL-1 and IL-18 systems, IL-18 had no effect on leukocytosis and did not increase

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.

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