ical analysis of paraffin sections of serotonin and ketanserin-treated cultures reveals another difference. The epidermis of the serotonin-treated cultures is covered by a thick border of keratin and shed epithelial cells, whereas the ketanserin-treated cultures hardly show any keratinization. As keratinization and shedding of epithelial cells can be seen as aspects of differentiation, our results indicate that serotonin enhances differentiation, whereas ketanserin inhibits differentiation.

We conclude that ketanserin is not only a 5-HT antagonist when acting on vascular tissue, but that it probably also has an immediate opposite effect on dermal and epidermal cells of the skin. It might be of some interest to test other 5-HT antagonists in our model. Experiments to demonstrate 5-HT receptors in fibroblasts are in progress. Up to now, the mechanism of action of these drugs on fibroblasts and epidermal cells is not clear. To find out more about the exact mechanism, more experiments will be carried out on organotypic cultures as well as on cell cultures of fibroblasts and of epithelial cells.

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Antibody to tumor necrosis factor (TNF) reduces endotoxin fever [1]

M. Nagai, T. Saigusa, Y. Shimada [a], H. Inagawa [b], H. Oshima [b] and M. Iriki

Department of Physiology, Medical University of Yamanashi, Tamaho, Nakakoma, Yamanashi 409-38 (Japan), [a] Tokyo Metropolitan Institute of Gerontology, Itabashi, Tokyo 173 (Japan), and [b] Department of Pharmaceutical Sciences, Teikyo University, Sagamiko, Tsukui, Kanagawa 199-01 (Japan)
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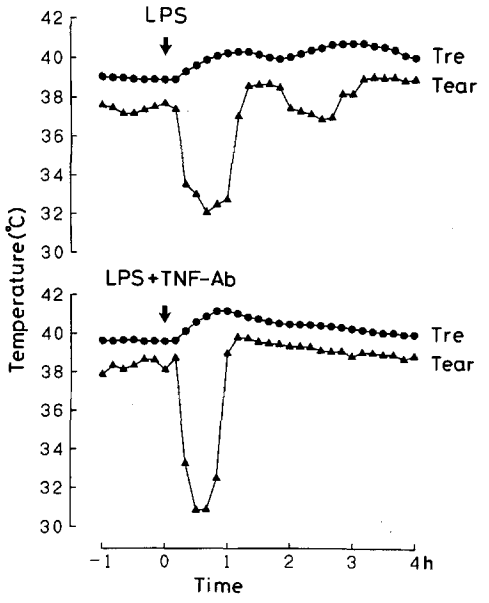
Summary. Antibody to tumor necrosis factor (TNF), injected intravenously, reduced endotoxin fever in the rabbit. The fever-reducing effect was apparent in the latter half of the febrile response.
Key words. Fever; lipopolysaccharide (LPS); tumor necrosis factor (TNF); monoclonal antibody to TNF.

It has long been postulated that pathogenesis of fever is essentially mediated by a heat-labile protein called endogenous pyrogen (EP) or leucocytic pyrogen (LP) which is synthesized and released by phagocytes in the face of infection by endotoxin [2]. The primary role of EP in fever genesis is to activate the synthesis of prostaglandins which are probably the final mediators acting on the regulatory center for body temperature to initiate fever [3,4]. A product of monocytes, interleukin-1 (IL-1) has recently been found to be identical with EP in its pyrogenicity and metabolic effects [5]. However, febrile responses to infection cannot be simply attributed to a PGE_2 induced by IL-1. Another leucocyte product, for example, interferon-α (IFN-α) can produce fever by its own pyrogenicity [6]. Also, tumor necrosis factor (TNF) has been reported to cause fever independently of its ability to produce IL-1 [7]. In the present experiments, we examined whether the production of TNF is substantially involved in fever caused by bacterial endotoxin, lipopolysaccharide (LPS), using an antibody to TNF.

Experiments were performed between December 1986 and June 1987. Male Japanese white rabbits, weighing 2.0–2.4 kg, were used. During 5 days before the experiments were done, animals had been adapted to settle down in the experimental chambers, in which the neck of the animal was loosely fixed, for at least 4 h a day. By this adaptation procedure, any effects of restraint-stress on body temperature were eliminated. Experiments were performed at an ambient temperature of 24.0 ± 1.0 °C. Rectal temperature, as being representative of body core temperature, was continuously measured with a thermistor probe inserted 10 cm into the rectum. Ear skin temperature was also measured with a thermistor probe. LPS derived from *E. coli* 0111:B4 (Difco Lab., USA) was resolved with sterile saline and injected into the ear vein. The dose of LPS was 100 ng/kg, and injection volume was 0.2 ml/kg.

Rabbit TNF was prepared as described previosuly [8,9]. Antibodies against rabbit TNF were prepared in the following way [10].

BALB/c mice were primed s.c. with 10–60 μg protein of 3000-fold purified TNF, which was emulsified in complete Freund's adjuvant. Mice received doses of 15–30 μg protein i.v. six times over a time period of five months. Three days after the last booster injection, spleen cells from the mice were fused with P3U1 (myeloma cell line) in the presence of polyethyleneglycol. Hybridoma cells which secreted antibodies to TNF were cloned by the limiting dilution method. Ascitic fluids were obtained after i.p. injection of hybridoma cells into BALB/c mice primed with pristane. Monoclonal antibody was purified by ammonium sulphate precipitation followed by ion exchange chromatography on DE52 (Whatman, England). Anti-TNF antibody activity was evaluated by neutralization of the cytotoxic activity of TNF on L929



Effects of monoclonal antibody to TNF (TNF-Ab) on LPS fever. In the upper figure, LPS (100 ng/kg) was injected i.v. at time zero. In the lower figure, LPS and TNF-Ab were simultaneously injected. Data from two different rabbits. Tre: rectal temperature; Tear: ear skin temperature.

cells[9,10]. TNF-Ab was injected i.v. at a dose of 40 µg/kg. This dose of TNF-Ab was determined from the concentration of serum TNF after LPS injection, and was considered to be sufficient to neutralize TNF activity.

The figure shows a time course of fever after LPS injection and the effect of TNF-Ab injected simultaneously with LPS. In the control animal, LPS caused a febrile response with a typical biphasic pattern. The two peaks of fever were associated with vasoconstriction of the ear skin vasculature as indicated by a fall in ear skin temperature. In the rabbit injected with TNF-Ab simultaneously with LPS, fever was still initiated, as in the control animal. However, the second peak of fever was not apparent, and consequently a decrease in ear skin temperature was not obvious, either. TNF-Ab reduced the fever during the latter half of the febrile response to LPS. TNF-Ab injected 60 min before LPS, and 60 and 120 min after LPS, had the same effect on the febrile response.

To evaluate this reduction of fever by TNF-Ab treatment quantitatively, we determined the fever index, i.e. the area under the fever curve after LPS injection. The fever index of every 1-h interval is shown in the table. TNF-Ab significantly reduced the fever index of the latter half of the febrile response independently of the time of antibody injection, which varied between 60 min before and 120 min after LPS (Student's t-test, $p < 0.05$ or $p < 0.01$). The percentage reduction of the fever index of 2–3 h and 3–4 h after LPS varied between 40 and 70%. However, the initiation of fever was not influenced by TNF-Ab treatment as shown by the fever index at 0–1 and 1–2 h. TNF-Ab itself, injected i.v., did not cause any significant changes in body temperature. Also, heated TNF-Ab (100 °C, 30 min) lost its ability to reduce fever.

The present results show that TNF activity is substantially involved in the febrile response to bacterial endotoxin, especially during the latter half of the response, i.e. from 2 h after endotoxin injection. These results are consistent with the fact that the TNF level in the rabbit serum reaches its maximum at 120 min postinjection of LPS[11]. LPS first activates macrophages to synthesize and release both IL-1 and TNF[8,12]. These two substances increase PGE_2 concentration in hypothalamic preparations with almost the same potency[7]. This explains why reduction of fever by TNF-Ab was not 100% but 40–70%.

Effects of monoclonal antibody to TNF (TNF-Ab), injected using different time-schedules, on LPS-induced fever. Fever index of every 1-h interval is shown. Data indicated by asterisk are statistically different from those for control animals (Student's t-test, * $p < 0.05$, ** $p < 0.01$).

		Fever index (°C · h) 0–1	1–2	2–3	3–4
Control	$\bar{x}$	0.66	1.31	1.49	1.32
	(n = 6) $S\bar{x}$	±0.02	±0.09	±0.11	±0.17
TNF-Ab	$\bar{x}$	0.66	1.24	1.00*	0.78
60 min before LPS	(n = 6) $S\bar{x}$	±0.06	±0.13	±0.19	±0.26
TNF-Ab	$\bar{x}$	0.76	1.23	0.83**	0.65*
simultaneously with LPS	(n = 5) $S\bar{x}$	±0.04	±0.06	±0.04	±0.18
TNF-Ab	$\bar{x}$	0.71	1.28	1.06	0.61*
60 min after LPS	(n = 4) $S\bar{x}$	±0.06	±0.15	±0.22	±0.27
TNF-Ab	$\bar{x}$	0.72	1.06	0.70**	0.42**
120 min after LPS	(n = 4) $S\bar{x}$	±0.03	±0.04	±0.10	±0.13

The residual fever is possibly due to IL-1 production that is induced by LPS. TNF does not play an essential role in initiating LPS-fever, but it is probably one of the substances which allow the animal to maintain fever.

The febrile response has recently been considered to be an adaptive response of the host organism against bacterial and viral infections. Indeed, animals prevented from achieving a febrile response show a higher rate of mortality[13,14]. Furthermore, Banet[15] has suggested that, although a higher febrile temperature is harmful in itself, an increase in metabolism during fever is beneficial, i.e., results in an increase in the survival rate of the animals[15]. On the other hand, TNF is a putative mediator of endotoxin shock. Indeed, immunization against TNF protects mice from the lethal effect of endotoxin[16]. Thus, to identify the role of TNF by its febrile and metabolic effects in the course of endotoxin fever, further experiments are required.

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