0014-4754/96/080774-04$1.50 + 0.20

Lack of tolerance development to tumor necrosis factor α inside the central nervous system

J. M. Goldbach, J. Roth, B. Störr and E. Zeisberger*

Physiologisches Institut, Klinikum der Justus-Liebig-Universität, Aulweg 129, D-35392 Gießen (Germany), Fax +49 641 702 4575
Received 8 November 1995; accepted 29 December 1995

Abstract. Tumor necrosis factor α (TNFα) was repeatedly microinfused into the lateral ventricle of guinea pig brains at a dose of 200 ng, 4 times within 150 min, at intervals of 3 days. In comparison to guinea pigs infused with solvent according to the same time schedule, the animals responded to TNFα with pronounced fevers. The quantity of the fever response was the same after each of the 4 microinfusions of TNFα. Three days after the last infusion of TNFα or solvent all animals received an intramuscular injection of bacterial lipopolysaccharide (LPS). The fever in response to LPS was the same in both groups. Thus, the reported development of tolerance to repeated systemic administration of TNFα[1–3] does not develop inside the blood-brain barrier. Also, the febrile response to LPS is not influenced by repeated central pre-treatment with TNFα, whereas repeated peripheral treatment does have an effect[3].
Key words. Tumor necrosis factor α; body temperature; fever; tolerance development; central nervous system; radiotelemetry; guinea pig.

TNFα is a multifunctional molecule, and has been identified as one of the mediators of detrimental effects caused by bacterial endotoxins in the infected host, such as hypotension, disseminated intravascular coagulation, tissue injury and wasting diathesis[4,5]. On the other hand, TNFα was successfully used in the treatment of cancer patients[2] although not all original hopes of benefit from TNFα for tumor therapy were fulfilled. Especially in view of the possible beneficial properties of TNFα, it is of interest to know whether the organism modifies its physiological responses to repeated administration of this cytokine. It has been reported that repeated administration of low doses of TNFα induced tolerance to the lethal effects of TNFα in mice[1,2]. In addition, we have shown recently that repeated infusion of a fever-inducing dose of TNFα into the circulation of guinea pigs resulted in a progressive attenuation of the febrile response[3], a phenomenon which can also be interpreted as a development of tolerance to TNFα. After peripheral injection of LPS into experimental animals, TNFα is induced and can be detected not only in the systemic circulation, but also in the brain of rats and guinea pigs[6–9]. Further, it has been shown that administration of TNFα into the ventricular system of the brain induces a febrile response in rats[10,11]. In the present study, we repeatedly microinfused a fever-inducing dose of TNFα into the lateral ventricle of the brain in guinea pigs, to find out whether tolerance to TNFα also develops inside the blood-brain barrier. Further, we investigated whether repeated central administration of TNFα modifies the febrile response to bacterial LPS, since we showed recently that the early phase of LPS-induced fever is suppressed after a partial development of tolerance to TNFα outside the blood-brain barrier[3].

* Corresponding author.

Materials and methods

Animals. This study was performed in 2 groups of 6 guinea pigs with a mean body weight of 370 ± 10 g at the time of surgery. The animals were housed in individual cages at 22 °C with a 12:12 h light-dark cycle (light off at 19.00 h).

Stereotaxic surgery. The animals were anesthetized with 100 mg/kg ketamine hydrochloride and 4 mg/kg xylazine. Guide cannulae were implanted bilaterally into the left and right lateral ventricles according to a stereotaxic atlas of the guinea pig brain[12]. The tips of the guide cannulae were located 8 mm in front of the interaural plane, 2.5 mm lateral to midline and 4.5 mm below the brain surface. Before the start of the experiments (see below) the microinfusion cannulae were introduced through the chronically implanted guide cannulae; the tip of the microinfusion cannulae protruded 0.5 mm beyond the guide. After surgery, the animals were allowed to recover for at least 8 days before experiments started.

Substances. Bacterial LPS was derived from *Escherichia coli* (O111:B4, Sigma Chemicals, St. Louis, MO, USA). It was dissolved in sterile pyrogen-free 0.9% saline at a concentration of 100 µg/ml. An amount of 20 µg/kg was injected into the thigh muscle.

Recombinant human (rh) TNFα, specific activity 20,000 units/μg (Biermann GmbH, Bad Nauheim, Germany) was used for intracerebroventricular infusions. The control group was infused with an equivalent volume of sterile pyrogen-free 0.9% saline, which was used as solvent. Two polyethylene tubes, each containing 100 ng TNFα dissolved in 15 μl solvent, were used for microinfusions into the lateral ventricles.

Experimental protocol. Microinfusions of TNFα or solvent into the lateral ventricles were performed in freely moving animals for 150 min by connecting the microinfusion cannulae to 5 ml glass syringes fixed to an infusion pump (type B, Braun, Melsungen, Germany) by polyethylene tubing (PE-10) filled with the infusion solutions (see above). The infusion speed was 0.1 μl/min, which corresponds to the flow of interstitial fluid in the brain[13]. The microinfusions were started between 10.00 and 11.00 h and repeated 4 times at intervals of 3 days. Another 3 days after the last of 4 infusions of TNFα or solvent all guinea pigs received intramuscular injections of 20 μg/kg LPS.

Measurement of body temperature. Abdominal temperature was measured with battery-operated biotelemetry transmitters (VM-FH-discs, Mini-Mitter Co., Sunriver, Oregon, USA) implanted intraperitoneally after stereotaxic surgery. Output (frequency in Hz) was monitored by a mounted antenna placed under each animal's cage (RA 1000 radio receivers, Mini-Mitter Co., Sunriver, Oregon, USA) and multiplexed by means of a BCM 100 consolidation matrix to an IBM personal computer system. A Dataquest IV data acquisition system (Data Sciences Inc., St. Paul, MN, USA) was used for automatic control of data collection and analysis. Body temperature was monitored and recorded at 5-min intervals. For the analysis and graphical documentation temperature data collected at 15-min intervals were used.

Evaluation and statistics. In graphs of the thermal responses to microinfusions with TNFα or solvent or to LPS-injections, the mean changes in abdominal temperature were plotted over time. At each time point abdominal temperature was expressed as mean ± SE. An analysis of variance (ANOVA) for repeated measures followed by Scheffe's post hoc test was used to compare thermal responses. The calculations were carried out on an Apple Macintosh computer using the software package StatView (Abacus Concepts, Berkeley, CA, USA). The mean fever indices, the integrated areas between thermal responses of febrile and normothermic animals, were expressed as a product of degrees Celsius and time in hours (for 8 hours after start of the microinfusions). The mean of all temperatures measured at 15-min intervals within 2 hours before the start of the microinfusion was calculated as the baseline temperature.

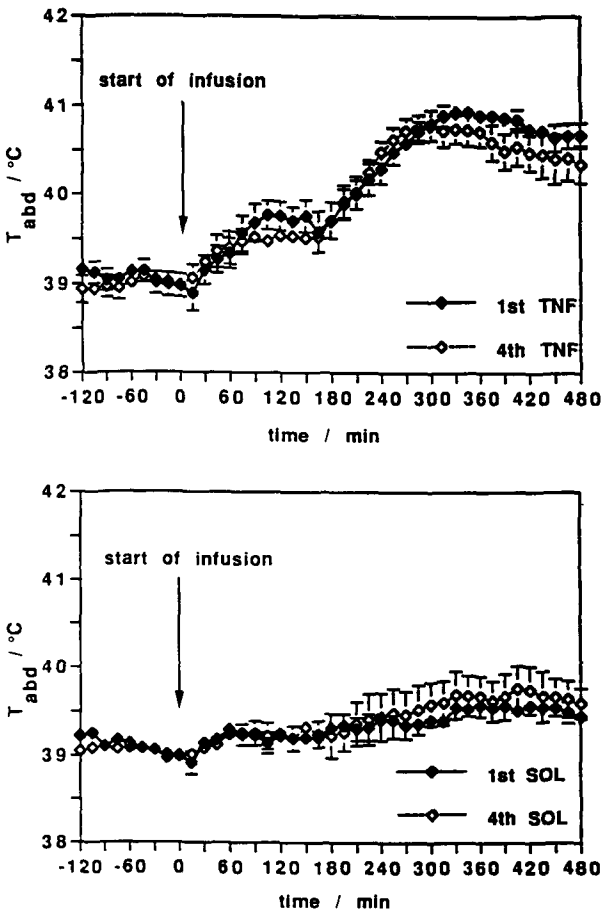

Figure 1. Effects of the first and the last of 4 repeated intracerebroventricular microinfusions of 200 ng TNFα (upper panel) or solvent (lower panel) on abdominal temperature in 2 groups of guinea pigs (N = 6 in each group). All symbols represent means ± SE.

Results

The effects of 4 repeated i.c.v. microinfusions of 200 ng TNFα or an equivalent volume of solvent on the abdominal temperature of guinea pigs were tested. The thermal responses to the 1st and the 4th microinfusions of TNFα or solvent are summarized in figure 1.

Each of the 4 microinfusions of TNFα evoked a pronounced rise of abdominal temperature, which was biphasic. The first phase was measured during the time of the microinfusion. The larger second phase of the thermal response, leading to abdominal temperatures of about 41 °C, started after the end of the microinfusion. The febrile responses to all of the 4 repeated i.c.v. administrations of TNFα were similar. The lower panel of figure 1 demonstrates that i.c.v. infusions of the same volume of solvent (15 μl into each of the lateral ventricles within 150 min) did not induce major changes of abdominal temperature.

Figure 2 shows the quantitative evaluation of all thermal responses to the repeated microinfusions of TNFα or solvent. The mean fever indices (±SE) of TNFα-infused

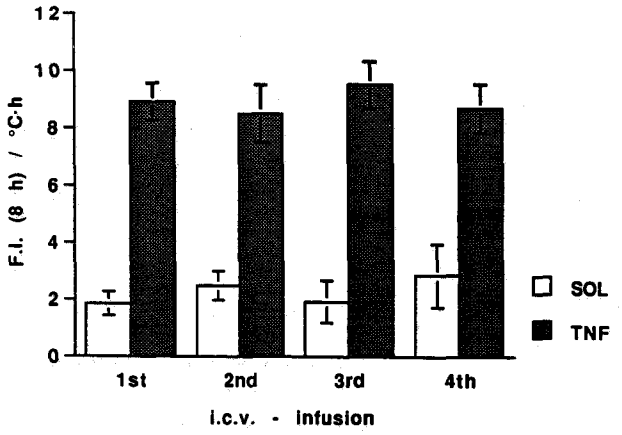

Figure 2. Integrated thermal responses (fever indices) calculated for each of the 4 microinfusion-experiments (TNFα or solvent) in two groups of guinea pigs (N = 6 in each group). Fever indices are presented as means ± SE.

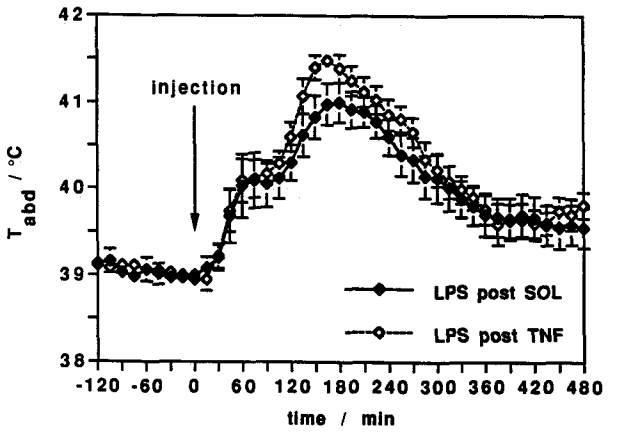

Figure 3. Fever responses to intramuscular injections of 20 μg/kg LPS in the two groups of guinea pigs shown in figure 2. LPS was administered 3 days after the last i.c.v. microinfusion of TNFα or solvent. Values are presented as means ± SE.

animals are represented by the shaded columns. The mean fever index was 8.92 ± 0.65 °C × h (for 8 h) in experiment 1, 8.51 ± 0.99 °C × h in experiment 2, 9.53 ± 0.81 °C × h in experiment 3 and 8.71 ± 0.84 °C × h in experiment 4. Thus, the thermal responses to each of the 4 i.c.v. microinfusions of TNFα were quantitatively the same.

In order to investigate whether the repeated pre-treatment with TNFα in the central nervous system alters the febrile response to LPS, all animals were injected with 20 μg/kg LPS three days after the last of 4 i.c.v. microinfusions of TNFα or solvent. The result of this experiment is shown in figure 3.

The febrile response to LPS was the same in animals which had received 4 central administrations either of TNFα or of solvent. Thus, in contrast to the effects of repeated infusions of TNFα outside the blood-brain barrier[1–3], tolerance to TNFα does not develop inside the blood-brain barrier, nor does the repeated central administration of TNFα influence LPS-induced fever in guinea pigs.

Discussion

It has been reported that an adaptation of the thermal response develops when TNFα[1–3] or interleukin-1 (ref. 14) are repeatedly administered outside the blood-brain barrier. This is the first study to investigate the effects of repeated i.c.v. infusions of a fever-inducing dose of TNFα on body temperature. In contrast to the attenuated responses after repeated peripheral administration, no tolerance developed to repeated central microinfusions of TNFα.

There is experimental evidence for separate mechanisms of fever induction inside and outside the blood-brain barrier in rabbits[15]. Moreover, the responses of rabbits to administration of pyrogens into the central nervous system were not altered in animals which were tolerant to exogenous[16] or endogenous[14] pyrogens administered peripherally. These phenomena have been called a 'lack of pyrogenic tolerance transmission between brain and periphery'[16]. Similarly, in this study a repeated central pre-treatment with an endogenous pyrogen (however, without tolerance development) did not influence the animals' responses to injections of LPS (fig. 3). This finding is interesting in that repeated peripheral pre-treatment with LPS suppressed the febrile response to peripheral infusion of TNFα[17], and repeated peripheral infusion of TNFα suppressed the first phase of fever induced by peripheral injection of LPS[3]. Thus, a cross-tolerance between LPS and TNFα, or vice versa, only manifests itself if both pyrogens are administered peripherally.

The main observation in the present study was that there is a lack of tolerance development per se inside the blood-brain barrier, at least in response to TNFα. In this context, it is of interest to summarize what is known about the induction of tolerance to TNFα outside the blood-brain barrier. In studies in mice[1,2] it could be demonstrated that the tolerance to the lethal effects of TNFα induced by repetitive pre-treatment with low doses of this cytokine was not due to the synthesis of antibodies (when the mice were made tolerant with human TNFα) or auto-antibodies (when the mice were made tolerant with murine TNFα). In these studies it became obvious, however, that the induction of tolerance was mediated by 55 kD TNF-receptor triggering[2]. Up to now it is not known which of the two receptors for TNFα (55 kD receptor or 75 kD receptor) is involved in the mediation of the effects of TNFα inside the central nervous system. If these effects, including the central induction of a febrile response, are mediated independently from the 55 kD TNF-receptor, this could explain the lack of tolerance development to TNFα inside the central nervous system. Finally, the fact that in the periphery and in the brain different types of cells

are the main targets for TNFα probably contributes to the lack of development of tolerance to this cytokine inside the central nervous system.

Acknowledgements. This study was supported by the Deutsche Forschungsgemeinschaft (DFG) project Ro 896/2-1.

1 Takahashi, N., Brouckaert, P., Bemelmans, M. H. A., Buurman, W. A., and Fiers, W., Cytokine *6* (1994) 235.
2 Takahashi, N., Brouckaert, P., and Fiers, W., Am. J. Physiol. *269* (1995) R398.
3 Goldbach, J. M., Roth, J., Störr, B., and Zeisberger, E., Am. J. Physiol. *270* (1996) R749.
4 Beutler, B., and Cerami, A., Nature *320* (1986) 584.
5 Beutler, B., and Cerami, A., New Eng. J. Med. *316* (1987) 379.
6 Gatti, S. S., and Bartfai, T., Brain Res. *624* (1993) 291.
7 Klir, J. J., Roth, J., Szelenyi, Z., McClellan, J. L., and Kluger, M. J., Am. J. Physiol. *265* (1993) R512.
8 Roth, J., Conn, C. A., Kluger, M. J., and Zeisberger, E., Am. J. Physiol. *265* (1993) R653.
9 Jansky, L., Vybíral, S., Pospíšilová, D., Roth, J., Dornand, J., Zeisberger, E., and Kamínková, J. Neuroendocrinology *62* (1995) 55.
10 Rothwell, N., J. Biosci. Rep. *8* (1988) 345.
11 Coelho, M. M., Luhesi, G., Hopkins, S. J., Pela, J. R., and Rothwell, N. J., Am. J. Physiol. *269* (1995) R527.
12 Luparello, T. J., Stereotaxic Atlas of the Forebrain of the Guinea Pig. Karger, Basel 1967.
13 Cserr, H. F., Neuroscience *22* (1987) S13.
14 Yamashiro, O., Morimoto, A., Sakata, Y., Watanabe, T., and Murakami, N., Am. J. Physiol. *264* (1993) R1180.
15 Morimoto, A., Murakami, N., Nakamori, T., and Watanabe, T., J. Physiol. (London) *383* (1987) 629.
16 Kozak, W., Soszynski, D., Szewczenko, M., and Bodurka, M., Experientia *46* (1990) 1010.
17 Roth, J., and Zeisberger, E., Am. J. Physiol. *268* (1995) R514.