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European Journal of Pharmacology 334 (1997) 249–254

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Changes of abdominal temperature and circulating levels of cortisol and interleukin-6 in response to intra-arterial infusions of tumor necrosis factor-α or tumor necrosis factor-β in guinea pigs

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Received 17 March 1997; revised 12 June 1997; accepted 11 July 1997

Abstract

The sister proteins tumor necrosis factor (TNF)-α and TNF-β share 35% of their amino acid sequence and a number, but not all, of their biological properties. In the present study we infused amounts of 5 μg/kg TNF-α, TNF-β (both preparations with identical bioactivities) or of solvent (0.9% sterile saline) into the circulation of guinea pigs and studied the effects on abdominal temperature, on the induction of endogenous formation of interleukin-6 and on levels of cortisol in plasma as a parameter of the activation of the hypothalamic–pituitary–adrenal axis. Infusion of TNF-α and TNF-β both resulted in identical circulating TNF-like-activities corresponding to an amount of about 7000 pg/ml. TNF-α induced a biphasic fever lasting for more than 6 h, while in response to TNF-β just the shorter first phase of fever (duration: 120 min) was measured. Circulating interleukin-6 (baseline level: 12–20 International Units (I.U.)/ml) and cortisol (baseline level: 70–120 ng/ml) increased about 6-fold during the first phase of the febrile response 60 min after the start of infusion with TNF-α or TNF-β. Thereafter interleukin-6 and cortisol declined again in response to TNF-β, but further increased after infusion with TNF-α to peak values measured 3 h after the start of infusion (interleukin-6: 258 ± 52 I.U./ml; cortisol: 790 ± 167 ng/ml). In animals infused with solvent abdominal temperature and interleukin-6 remained at the baseline values, just cortisol increased slightly. The results demonstrate that TNF-α is a much stronger inducer of fever and interleukin-6 production or of HPA-axis activation than TNF-β in so far as all the investigated responses can be measured for prolonged time in response to TNF-α. © 1997 Elsevier Science B.V.

Keywords: TNF-α (tumor necrosis factor-α); TNF-β; Acute-phase-response; Fever; Interleukin-6; Hypothalamic–pituitary–adrenal axis; Cortisol

1. Introduction

The cytokines TNF-α and TNF-β share about 35% of their amino acid sequence and display competitive binding at the same receptors (Pennica et al., 1984; Fiers, 1991; Aggarwal, 1992). Therefore it is not surprising that some biological properties of TNF-α and TNF-β are identical, for example the cytotoxic effect on some cell lines (Gray et al., 1984; Tomasovic et al., 1992) including the mouse fibrosarcoma cell line WEHI 164 clone 13 which we use to measure TNF-like bioactivity (see below). On the other hand, there are profound differences between TNF-α and TNF-β. Both cytokines are produced by different cell types, and distinct inducers stimulate endogenous production and release of TNF-α and TNF-β (Aggarwal, 1992). Studies investigating the effects of exogenous administrations of TNF-α or TNF-β in vivo were leading, in part, to conflicting results. While pyrogenic effects of TNF-α and TNF-β were observed in rabbits (Kapas and Krueger, 1992; Watanabe, 1992), opposite effects of TNF-α and TNF-β on sympathetic efferent nerves to brown adipose tissue and rectal temperature were reported in rats (Holt et al., 1989).

In 2 of our previous studies, we investigated the influence of intra-arterial infusions of TNF-α on abdominal temperature in guinea pigs (Roth et al., 1994; Goldbach et al., 1997). In both studies we infused an amount of TNF-α comparable to the endogenous TNF-like-activity measured after injection of a fever-inducing dose of bacterial lipopolysaccharide and observed a biphasic rise of body core temperature. In the present study, we compared the effects of intra-arterial infusions of TNF-α or TNF-β (both preparations with identical bioactivities) on body temperature,

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induction of interleukin-6 and circulating levels of cortisol as a sign of activation of the hypothalamic–pituitary–adrenal axis. These measurements should enable us to state if comparable circulating bioactivities of TNF-α or TNF-β are able to elicit similar or distinct biological responses in vivo.

2. Materials and methods

2.1. Animals

This study was performed in 3 groups of male guinea pigs ($N = 6$ in each group) with a body weight of 378 ± 10 g at the beginning of the experiments. The animals were housed in individual cages at 22°C with a 12:12 h light/dark cycle (light off at 7.00 p.m.).

2.2. Surgery

At least one week before the start of the experiments, the animals were chronically implanted with intra-arterial catheters. The guinea pigs were anesthetized with 100 mg/kg ketamine hydrochloride and 4 mg/kg xylazin. Polyethylene catheters were inserted through the left carotid artery until reaching the aortic arch. The distal ends of the catheters were tunnelled subcutaneously to the interscapular region of the back where they emerged through the skin as described previously (Roth et al., 1993). After implantation, the catheters were flushed with sterile heparinized saline and closed by heating. One day before the experiment we tested if blood sampling was possible by opening the catheter followed by slow aspiration of blood. Then the catheters were flushed again with sterile heparinized saline.

2.3. Experimental procedure

Recombinant human (rh) TNF-α and TNF-β, specific activity of both substances 20,000 U/μg (Biermann, Bad Nauheim) were used for intra-arterial infusions. The level of endotoxin in both preparations was lower than 0.1 ng per 1 μg of the cytokine. This level of endotoxin is unable to induce fever or any other component of the acute-phase response in guinea pigs. The low endotoxin sensitivity of guinea pigs requires endotoxin doses in the range of several μg to evoke the acute-phase response. Therefore all observed biological responses were due to the biological activity of TNF-α or TNF-β. An amount of 5 μg/kg TNF-α or TNF-β was dissolved in 2 ml sterile pyrogen-free 0.9% saline before the start of the infusion. In previous studies (Roth et al., 1993; Roth et al., 1994; Goldbach et al., 1997) we had determined the dose of TNF-like bioactivity which had to be infused into the systemic circulation of guinea pigs to simulate the circulating TNF-activity caused by a fever-inducing dose of bacterial lipopolysaccharide. The kinetics of TNF-like bioactivity in plasma after an intra-arterial infusion of about 40,000 bioactive units of TNF-α or TNF-β and the endogenous release of TNF-like activity in response to lipopolysaccharide are almost identical. To investigate a role of TNF-bioactivity in the development of fever, we selected the dose of TNF-α or TNF-β with the bioactivity mentioned above.

Intra-arterial infusions of TNF-α, TNF-β or solvent were performed in conscious guinea pigs freely moving in the cages, in which they were housed according to the following schedule. The distal end of the implanted catheter (see above) was connected by an adapter and polyethylene tubing to a 5 ml glass syringe fixed to an infusion pump (type B, Braun, Melsungen). 5 μg/kg rh TNF-α or rh TNF-β dissolved in 2 ml sterile pyrogen free saline or the same volume of solvent alone were infused into the animals' circulation within 45 min. During the experiment, single blood samples (0.6 ml) were collected 60 min before as well as 60 and 180 min after the start of infusion. The samples were slowly (within 1 min) drawn into a sterile syringe, put into a polypropylene tube and immediately centrifuged. Plasma was frozen at −70°C for later determination of TNF-like and interleukin-6-like activities as well as cortisol.

2.4. Measurement of body temperature

Abdominal temperature was measured by use of battery operated biotelemetry transmitters (VM-FH-discs, Mini-Mitter, Sunriver, OR) implanted intraperitoneally after placement of the intra-arterial catheter or separately in animals not equipped with catheters. Output (frequency in Hz) was monitored by a mounted antenna placed under each animal's cage (RA 1000 radioreceivers, Mini-Mitter, Sunriver, OR) and multiplexed by means of a BCM 100 consolidation matrix to an IBM personal computer system. A Dataquest IV data acquisition system (Data Sciences, St. Paul, MN) 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 of 15 min intervals were used.

2.5. Bioassays for TNF and interleukin-6

Determination of TNF was performed by a bioassay based on the cytotoxic effect of TNF on the mouse fibrosarcoma cell line WEHI 164 subclone 13 (Espevic and Nissen-Meyer, 1986). The assay was performed in sterile 96 well microtiter plates. Serial dilutions of biological samples, or different concentrations of TNF-standard (code 88/532, National Institute for Biological Standards and Control, South Mimms) were incubated for 24 h in wells that had been seeded one day earlier with 50,000 actinomycin D-treated WEHI cells. The number of surviving cells after 24 h was measured by use of the dimethylthiazol–diphenyl tetrazolium bromide (MTT) colorimetric as-

say (Holt et al., 1991) with an ELISA-reader at 550 nm wavelength (MR 7000, Dynatech, Denkendorf).

Determination of interleukin-6 was performed by a bioassay based on the dose dependent growth stimulation of interleukin-6 on the B9 hybridoma cell line (Aarden et al., 1987). The assay was performed in sterile 96 well microtiter plates. In each well 5,000 B9 cells were incubated for 72 h with serial dilutions of biological samples or with different concentrations of interleukin-6 standard (code 89/548, National Institute for Biological Standards and Control, South Mimms). The number of cells in each well was measured by use of the MTT assay (see above).

The WEHI cells and the B9 cells were kindly provided by Dr. Stephen Hopkins, University of Manchester, UK.

2.6. Radioimmunoassay for cortisol

Cortisol levels were determined by a specific radioimmunoassay (DPC Biermann, Bad Nauheim). 25 μl standard- or test-sample and 1000 μl cortisol (^{125}I) solution were incubated for 45 min at 37°C in tubes coated with cortisol-antibodies. The radioactivity of bound cortisol was determined by a gamma-counter (model Cobra QC, Canberra Packard, Frankfurt). Cross-reactivities with other natural steroids was 0–1.5%; Intra-assay and inter-assay coefficients of variation were 2–5%.

2.7. Evaluation and statistics

In graphs of the thermal responses to intra-arterial infusions of TNF-α, TNF-β or 0.9% NaCl, the mean changes in abdominal temperature were plotted over time. At each time point abdominal temperatures were expressed as means ± SEM. 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). Circulating levels of TNF-α, interleukin-6 and cortisol were compared by Student's *t*-tests. Because the values for cytokine concentrations are not normally distributed, a log-transformation of the cytokine values was performed for the *t*-tests.

3. Results

The effects of intra-arterial infusions of TNF-α, TNF-β or solvent (0.9% sterile pyrogen-free saline) on abdominal temperature are summarized in Fig. 1.

In response to infusion of TNF-α, a biphasic increase of abdominal temperature was measured, the first phase from 15–120 min and a second phase from 120–360 min after the start of infusion. TNF-β just evoked a thermal response corresponding to the first phase of TNF-α-induced fever, while the second phase did not become manifest in response to TNF-β ($p < 0.001$, ANOVA). Intra-arterial infusion of solvent had no apparent influence on body temperature of guinea pigs.

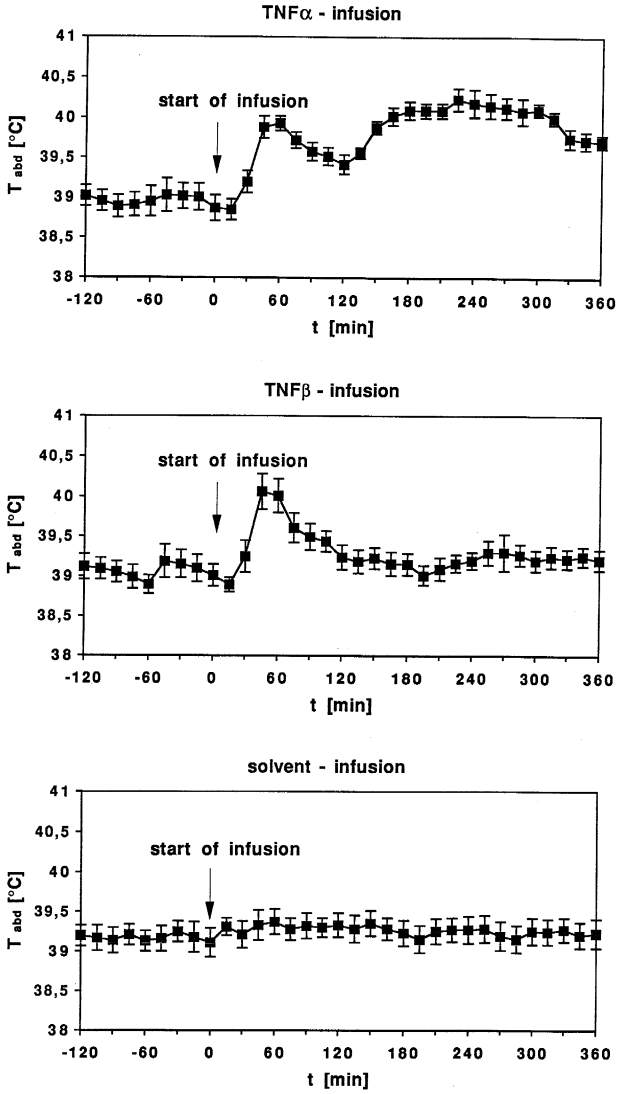

Fig. 1. Abdominal temperature of guinea pigs in response to intra-arterial infusions of 5 μg/kg TNF-α (upper part), 5 μg/kg TNF-β (middle part) and sterile 0.9% saline (lower part). Symbols represent means ± S.E.M., $N = 6$ in each group.

Circulating TNF-like bioactivity, interleukin-6-like bioactivity and cortisol were measured 60 min before as well as 60 and 180 min after the start of intra-arterial infusions of TNF-α, TNF-β or solvent. The results of these measurements are shown in Fig. 2.

One hour after the start of the infusions a mean circulating TNF-like-activity of 6980 ± 740 pg/ml was measured in animals infused with TNF-α, while the corresponding value was 7530 ± 1130 pg/ml in animals infused with TNF-β. These levels declined within the next 2 h to 1420 ± 180 pg/ml in response to infusions of TNF-α and to 350 ± 20 pg/ml in response to infusions of TNF-β. These data show that circulating peak-activity for both

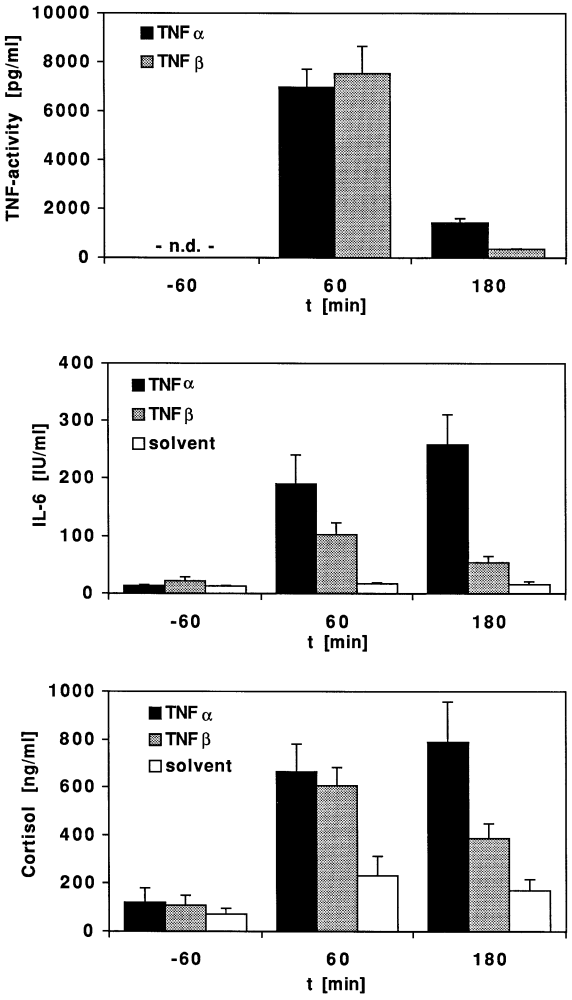

Fig. 2. Upper panel: Circulating TNF-like bioactivity in response to intra-arterial infusions of 5 μg/kg TNF-α or 5 μg/kg TNF-β. Each column represents means ± S.E.M. from 6 animals. In response to infusions of 0.9% sterile saline no TNF-bioactivity was detected. Middle panel: Circulating interleukin-6 bioactivity before and after intra-arterial infusions of 5 μg/kg TNF-α, 5 μg/kg TNF-β or sterile 0.9% saline. Each column represents means ± S.E.M. from 6 animals. Lower panel: Circulating levels of cortisol before and after intra-arterial infusions of 5 μg/kg TNF-α, 5 μg/kg TNF-β or sterile 0.9% saline. Each column represents means ± S.E.M. from 6 animals.

cytokines, measured briefly after the end of in fusion, was identical ($p = 0.776$). The significantly lower circulating TNF-bioactivity of TNF-β-infused animals measured 180 min after start of infusion ($p < 0.0001$) indicated a more rapid clearance of this cytokine from the systemic circulation. In animals receiving infusions of solvent as well as 60 min before the start of infusions of TNF-α or TNF-β, no TNF-like-activity was detected in arterial plasma. The effects of the infusions of TNF-α, TNF-β or solvent on circulating bioactive interleukin-6 are summarized in the middle panel of Fig. 2.

In contrast to other cytokines, small amounts of bioactive interleukin-6 are detectable under basal conditions. One hour before the infusions started, mean activities of 12–20 I.U./ml interleukin-6 were measured. One hour after the start of the infusions the activity of interleukin-6 in plasma rose to 190 ± 50 I.U./ml in response to TNF-α and to 103 ± 20 I.U./ml in response to TNF-β. Both values were significantly higher than the corresponding circulating interleukin-6 level after infusion of the solvent, which was 16 ± 2 I.U./ml ($p < 0.0001$ for TNF-α versus solvent; $p = 0.0002$ for TNF-β versus solvent). Three hours after the start of the infusions bioactive interleukin-6 in plasma further increased to 260 ± 53 I.U./ml in guinea pigs infused with TNF-α and decreased again to 54 ± 10 I.U./ml in those animals having received infusions of TNF-β. These values were significantly different from each other ($p = 0.0012$) and in the TNF-α infused animals circulating interleukin-6 was also higher than the corresponding interleukin-6 level in guinea pigs infused with solvent ($p = 0.0099$). At that time interleukin-6 in plasma was not significantly different in animals infused with TNF-β or solvent ($p = 0.0996$).

Circulating levels of cortisol are shown in the lower panel of Fig. 2. One hour before the infusions started, mean amounts of 70–120 ng/ml cortisol were measured in arterial plasma of guinea pigs. These values increased to 665 ± 118 ng/ml (infusion of TNF-α), 606 ± 75 ng/ml (infusion of TNF-β) and to 230 ± 82 ng/ml (infusion of solvent) one hour after the start of infusion. At that time circulating cortisol was significantly higher ($p < 0.01$) in response to TNF-α or TNF-β when compared to the corresponding value of animals infused with solvent. Three hours after the start of infusion, cortisol in plasma further increased to 790 ± 168 ng/ml in response to TNF-α, and declined again to 387 ± 62 ng/ml in response to TNF-β, respectively, to 169 ± 45 ng/ml in response to solvent. At that time circulating cortisol was significantly higher in animals infused with TNF-α when compared to the corresponding values measured in guinea pigs infused with TNF-β ($p < 0.05$) or solvent ($p < 0.001$). The data of our cortisol measurements confirmed the ability of TNF-α and TNF-β to activate the hypothalamic–pituitary–adrenal axis. The increase of circulating cortisol in response to infusion of solvent ($p < 0.05$, compared to the pre-infusion value) may have reflected a general stress response.

4. Discussion

The major sources of TNF-α production are activated monocytes, macrophages and a number of other cell types, while the production of TNF-β seems to be restricted to lymphocytes. Also the induction of both cytokines is regulated differently. Release of TNF-α is induced by a variety of agents which are without effects on TNF-β induction, for example lipopolysaccharide from gram-negative bacteria (Aggarwal, 1992). In spite of their abilities to activate both TNF-receptors the biological properties of TNF-α

and TNF-β are not identical (see introduction). TNF-α has been identified as one of the most important endogenous mediators of the host response to infection (Beutler and Cerami, 1989). Several components of the so called acute-phase-response can be evoked by administration of TNF-α including fever (Dinarello et al., 1986; Blatteis et al., 1991; Kluger, 1991; Roth and Zeisberger, 1995; Stefferl et al., 1996), induction of endogenous formation of interleukin-6 (Fong et al., 1989; Roth et al., 1994) and activation of the hypothlamic–pituitary–adrenal axis (Bernardini et al., 1990; Ebisui et al., 1994). The aim of this study was to investigate if comparable amounts of TNF-β are able to stimulate these components of the acute-phase-response with the same strength as TNF-α.

As shown in Figs. 1 and 2, the duration of fever and of the interleukin-6 or cortisol responses were of short duration after infusion of TNF-β and sustained for several more hours after infusion of TNF-α. What might be the reasons for the quantitative differences in the observed effects? One possible explanation was provided by our own measurements. TNF-like-bioactivity circulating in response to infusions of TNF-α or TNF-β reached similar values after the end of infusion. The declining level of TNF-bioactivity measured 2 h later was, however, significantly lower in response to infusions of TNF-β, indicating that TNF-β might be cleared faster from the circulation or inactivated more effectively than TNF-α. This is surprising in so far as the molecular size of TNF-β is higher than that of TNF-α, and TNF-α is more susceptible to proteases than TNF-β (Aggarwal, 1992). However, it has been demonstrated that TNF-α or TNF-β participate in self-induction mechanisms meaning that administration of exogenous TNF causes production of endogenous TNF (Phillip and Epstein, 1986). Therefore, part of the TNF-like activity measured in vivo (see upper part of Fig. 2) could be due to endogenous formation of TNF. If the exogenously-added TNF-α induced more endogenous TNF-like activity than TNF-β, this might explain the higher TNF concentration in TNF-α-infused animals measurable 3 h after the start of the infusion. Such a mechanism could also be involved in the observed differences of interleukin-6 and cortisol production. An alternative explanation for the observed effects is that receptor-activation by TNF-α is more effective and powerful than by TNF-β or that the signal transduction mechanisms differ substantially resulting in the greater strength of the signal mediated by TNF-α. Distinct pathways of signal transduction would provide a reasonable explanation for the antagonistic effects of TNF-α and TNF-β in several experimental models (Locksley et al., 1985; Beran et al., 1987; Oster et al., 1987; Holt et al., 1989). In this context, it has to be considered that we used human TNF-α or TNF-β in guinea pigs. It has been demonstrated that human TNF is a selective agonist of the murine 55 kDa but not of the murine 75 kDa TNF-receptor (Lewis et al., 1991; Takahashi et al., 1995). Possible selective species-specificity of interaction between human TNF-α or TNF-β with the TNF-receptors of guinea pigs could therefore also contribute to the observed differences. Support for this hypothesis is given by a recent study in rats showing that human or murine TNF-α cause different physiological responses in rats (Stefferl et al., 1996).

However, concerning fever, interleukin-6-induction and activation of the hypothalamic–pituitary–adrenal axis, the results of our study show that TNF-α and TNF-β influence these parameters in the same direction, although with different strength. One reason for the different strength of TNF-α or TNF-β in inducing the observed effects in vivo may be due to potential differences of TNF-α and TNF-β in inducing other very important endogenous pyrogens such as interleukin 1β (Dinarello et al., 1986) or interferon-γ (Wherry et al., 1991). Both cytokines may, however, play a role as endogenous mediators in the host's responses to infectious, inflammatory or mitogenic stimuli.

Acknowledgements

The study was supported by the Deutsche Forschungsgemeinschaft. We thank Dr. Stephen Hopkins (University of Manchester, UK) for providing us with the WEHI 164 subclone 13 and the B9 cell lines and teaching us important details of the cytokine assays.

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