The cardiodepressant and vasodepressant effects of tumour necrosis factor in rat isolated atrial and aortic tissues

¹R. Foulkes & S. Shaw

Immunomodulation Biology, Celltech Research, 216 Bath Road, Slough, SL1 4EN

1 The ability of recombinant human tumour necrosis factor- α (rec huTNF) to elicit cardiodepressor and vasodepressor effects in rat isolated tissues was investigated.

2 rec huTNF (3×10^{-11} – 3×10^{-8} M) administered directly to the organ bath, caused a concentration-dependent relaxation of the isoprenaline-induced inotropic response in electrically stimulated rat left atria. This occurred within 20 min of administration. In contrast, rec huTNF was without effect on the chronotropic response to isoprenaline in isolated spontaneously beating atria.

3 rec huTNF ($1 \mu\text{g kg}^{-1}$) was also given systemically to rats and the atria studied *in vitro*. Only 60 min of rec huTNF pretreatment was sufficient to cause a marked attenuation of the isoprenaline-induced inotropic response. This effect was not further augmented when rats were pretreated with rec huTNF for 24 h.

4 In isolated aortic rings taken from rats 60 min after rec huTNF ($1 \mu\text{g kg}^{-1}$, i.v.) administration, there was no effect seen on the constriction induced by phenylephrine in either endothelium-intact or denuded tissues. In addition, any responses to L-arginine or N^G-nitro-L-arginine methyl ester (L-NAME) administration were unaffected by rec huTNF pretreatment.

5 In aortic rings taken from rats 24 h after rec huTNF administration, the phenylephrine-induced constriction was significantly attenuated in tissues with an intact endothelium. Furthermore, the relaxation to subsequent L-arginine administration was greater in these tissues than in those from saline-treated rats. In addition, in both endothelium-intact and denuded tissues, the vasoconstrictor response to L-NAME (10^{-3} M) was significantly augmented.

6 These data suggest that rec huTNF possesses both cardiodepressant properties with a rapid onset of action and vasodepressant properties with a slow onset of action. The latter could be mediated through the induction of a non-constitutive form of the NO-synthase enzyme present within the vascular wall.

Keywords: Tumour necrosis factor; nitric oxide; myocardial depression; vasodepression

Introduction

In animals given endotoxin or gram negative bacteria, one of the primary mediators of the ensuing cardiovascular shock state is tumour necrosis factor- α (TNF), a cytokine released from activated monocytes and macrophages as well as other cell types (Beutler & Cerami, 1987). Indeed monoclonal antibodies raised against TNF can improve survival and ameliorate some of the cardiovascular manifestations associated with septic shock in a number of animal species (Beutler *et al.*, 1985; Mathison *et al.*, 1988; Hinshaw *et al.*, 1990). Furthermore, TNF, like lipopolysaccharide (LPS), can induce the expression and synthesis of a form of NO-synthase in macrophages and other cells (Jorens *et al.*, 1991), including smooth muscle (Busse & Mulsch, 1990) and endothelial cells (Kilbourn & Belloni, 1990).

Cardiovascular shock states such as haemorrhagic or endotoxic shock resulting from septicaemia are characterized by marked hypotension, myocardial depression and acidosis often leading to the failure of one or more organs. In man and animal models of these disease states, depression of cardiac performance has been associated with the appearance of a circulating factor which can itself suppress cardiac function in *in vitro* and *in vivo* bioassays (Goodyear, 1967; Lefer, 1970; Lefer & Rovetto, 1970). Although thought to be of peptide origin, the precise nature of this substance(s) remains unknown. In addition, there is growing evidence that the hypotension associated with endotoxin-induced shock states in animals is at least partly dependent on the L-arginine-NO

pathway. Indeed, this hypotensive state can be readily reversed by the administration of inhibitors of nitric oxide synthase (Wright *et al.*, 1992). Furthermore, in *in vitro* studies in aortic tissues taken from rats previously given LPS, a reduced vascular responsiveness to phenylephrine has been shown to be associated with the induction of a non-constitutive form of NO-synthase in the tissues (Fleming *et al.*, 1991). However, given the multifarious nature of cytokines and hormones released in response to endotoxin administration, it is unclear which, if any, of these have inherent cardiac and vascular depressive properties.

Hence, the present study investigated whether some of the cardiac and vascular depressant effects known to be associated with LPS administration could also be seen with TNF treatment. This was addressed by studying the effects of recombinant human TNF (rec huTNF) on isolated cardiac and vascular preparations from rats.

Methods

1 Effects of rec huTNF on rat isolated left and right atria

Male Sprague Dawley rats (300–350 g) were killed by a blow to the head and cervical dislocation, the atria rapidly removed and each mounted on electrodes in a 100 ml organ bath. The upper portion of each atrium was connected to a Grass FT03 force-displacement transducer and isometric tension was recorded on a Gould TA550 recorder. The organ baths were filled with Krebs solution of the following com-

¹ Author for correspondence.

position (in mM): NaCl 118, NaHCO₃ 25, D-glucose 11, KCl 4.7, CaCl₂ 2.5, KH₂PO₄ 1.2 and MgSO₄·7H₂O 1.2, maintained at 37°C and gassed continuously with a mixture of 95% O₂ and 5% CO₂. Left atria were electrically stimulated by a Grass stimulator using a supra-maximal voltage at 90 Hz with a pulse duration of 5 ms whereas the right atria beat spontaneously.

Tissues were allowed to equilibrate for 60 min under a resting tension of 1 g following which a single bolus of isoprenaline (10⁻⁶ M) was added to the bath to sensitize the tissues (previous pilot studies have shown that after this initial sensitization period up to 4 concentration-response curves (CRCs) to isoprenaline can be carried out without the development of tachyphylaxis). Following repeated washing a CRC to isoprenaline (10⁻¹⁰–10⁻⁶ M) was performed with a 3 min dosing cycle. The tissues were then washed and a second CRC was performed according to one of the following treatments: (1) CRC to isoprenaline only, (*n* = 6); (2) CRC to isoprenaline in the presence of rec huTNF (3 × 10⁻¹²–3 × 10⁻⁸ M) given 15 min earlier (*n* = 6); (3) CRC to isoprenaline in the presence of rec huTNF (3 × 10⁻¹⁰ M) and the additional presence of a specific anti-rec huTNF monoclonal antibody (CB0010, 3 × 10⁻⁷ M or 3 × 10⁻⁹ M) given 15 min before rec huTNF (*n* = 6). In some tissues (*n* = 6), an isotype-matched control antibody, MOPC21 (3 × 10⁻⁷ M) was given instead of CB0010. Neither of these antibodies alone had any effect on the second isoprenaline CRC.

In addition, the effect of prior rec huTNF administration on the inotropic response to 1-isobutyl, 3-methylxanthine (10⁻⁸–10⁻⁴ M) was also studied (*n* = 6).

2 Effect of systemic (i.v.) administration of rec huTNF on rat isolated atria

Male Sprague Dawley rats (300–400 g) were briefly anaesthetized with halothane (2% in O₂) and given either rec huTNF (1 µg kg⁻¹ in 1 ml kg⁻¹ 0.9% saline bolus, *n* = 6) or 1 ml kg⁻¹ 0.9% saline (*n* = 6) via the tail vein. Rats were killed at either 1 h or 24 h post administration, the atria removed and prepared as before. Following the equilibration and isoprenaline sensitization, a single CRC to isoprenaline (10⁻¹⁰–10⁻⁶ M) was obtained. Previous studies in this laboratory have shown this dose of rec huTNF to be non-hypotensive and non-lethal in this species.

3 Effects of systemic rec huTNF pretreatment on rat isolated aortic rings: interaction with the L-arginine-NO pathway

rec huTNF (1 µg kg⁻¹) or 0.9% saline was administered as a single bolus (1 ml kg⁻¹) via the tail vein to male Sprague Dawley rats (300–400 g). Animals were killed either 1 h or 24 h later and the aortae rapidly removed. Aortic rings (2–3 mm diameter and 1–2 mm in length, *n* = 12) were mounted in 10 ml organ baths, connected to a Grass FT03 transducer and isometric tension recorded on a Grass polygraph. Tissues were bathed in Krebs solution and equilibrated under the optimal resting tension for the tissues (around 1.5 g). In half of the preparations (*n* = 6) the endothelium was removed by gently rubbing the lumen with the barrel of a syringe needle. All experiments were performed in the presence of 10⁻³ M indomethacin to remove any possible influence of cyclo-oxygenase-mediated events.

Rings were precontracted with 10⁻⁴ M phenylephrine and the presence of functional endothelium was assessed by observing the vasodilator response to 10⁻⁶ M acetylcholine (ACh); rings showing no relaxation or a small constriction were considered endothelium-denuded and those showing more than 40% relaxation were considered as endothelium-intact tissues. This was confirmed at the end of the experiment by histological examination. Following repeating washing and a 90 min stabilization period a cumulative CRC to

phenylephrine (10⁻⁹–10⁻⁴ M) was performed with a 5 min dosing cycle; tissues were then repeatedly washed and re-equilibrated. All tissues were then constricted to 70% of maximal constriction with phenylephrine and a cumulative CRC to either D- or L-arginine (10⁻⁶–10⁻³ M) was carried out with a 15 min dosing cycle. After a further washing and stabilization period, tissues were again constricted to 70% of maximum with phenylephrine but this time in the presence and absence of N^G-nitro-L-arginine methyl ester (L-NAME, 10⁻³ M) given 15 min previously and the change in tension measured.

Data analysis

All data are presented as means ± s.e.mean. In experiment (1), CRCs were compared by calculating the EC₅₀ values for the pairs of curves and performing a paired *t*-test. To compare relative shifts at each concentration of rec huTNF, dose ratios were calculated and these were compared by one-way ANOVA. In experiment (2), mean EC₅₀ values were compared by an unpaired *t* test. In experiment (3), mean EC₅₀ values for the phenylephrine constriction curves and the mean changes in tension following L-NAME administration were compared by one-way ANOVA.

Materials

The monoclonal antibodies CB0010 and MOPC21 were manufactured by Celltech. CB0010 specifically neutralizes rec huTNF whereas MOPC21 is a nonspecific isotype matched control antibody. rec huTNF was purchased from Bissendorf Biochem (Hanover, Germany) and all other drugs from Sigma (Poole, UK). Both antibodies and rec huTNF were dissolved in saline before use.

Results

1 Effect of rec huTNF on rat isolated left and right atria

Isoprenaline, administered alone, caused a concentration-dependent increase in force (up to +0.742 ± 0.13 g) and rate of contraction (up to 440 ± 20 beats per min) in isolated left and right atria, respectively, with a mean EC₅₀ of 6 ± 10⁻⁹ M. Pilot studies indicated that up to 4 CRCs to isoprenaline could be performed on the same tissue without the development of tachyphylaxis.

In left atria, following the first CRC to isoprenaline, 20 min incubation with rec huTNF did not significantly alter the basal contractility. In the presence of rec huTNF, there was concentration-dependent rightward shift in the isoprenaline CRC, the threshold being 3 × 10⁻¹¹ M rec huTNF (Figure 1a) and the maximum seen at 3 × 10⁻⁹ M (Figure 1b). Although the CRC shifts were not large (maximum dose-ratio = 2.7 ± 0.5; *P* < 0.01), they were parallel and not accompanied by a decrease in the maximum isoprenaline response. It should be noted that incubation with rec huTNF for periods up to 60 min did not result in a greater shift in the isoprenaline CRC.

This effect was specific for rec huTNF, since in the presence of a neutralising monoclonal antibody raised against rec huTNF (CB0010, 3 × 10⁻⁷ M), the rec huTNF-mediated cardiodepressive response was prevented (dose-ratio = 1.08 ± 0.05). Furthermore, neither a lower concentration (3 × 10⁻⁹ M of CB0010 (dose-ratio = 2.62 ± 0.6, *P* < 0.05) nor a non-specific murine monoclonal antibody (MOPC21, 3 × 10⁻⁷ M, dose ratio = 2.0 ± 0.17, *P* < 0.05) were able to modify the rec huTNF response.

The effect of rec huTNF on the inotropic response in left atria was not restricted to β-receptor-mediated events since similar shifts in CRCs were seen when the phosphodiesterase inhibitor, IBMX, was used instead of isoprenaline (dose-

Figure 1 The effect of recombinant human tumour necrosis factor- α (rec huTNF) administration on the isoprenaline-induced inotropic response in rat isolated left atria. A concentration-response curve to isoprenaline was first obtained ($\circ$) and, following washing, rec huTNF was administered directly to the bath at either 3×10^{-11} M (a) or 3×10^{-9} M (b) and a second isoprenaline curve obtained ($\bullet$). Values are mean with s.e.mean shown by vertical lines. $n = 6$ for each group.

Figure 2 Effect of systemic recombinant human tumour necrosis factor- α (rec huTNF) pretreatment on isoprenaline-induced inotropic responses in rat isolated left atria. Rats received either saline (1 ml kg $^{-1}$, i.v., $\circ$) or rec huTNF (1 μ g kg $^{-1}$, i.v., $\bullet$) 60 min (a) or 24 h (b) before they were killed and the atria removed. A single isoprenaline curve only was obtained on each tissue. Values are means with s.e.mean shown by vertical lines; $n = 6$ for each group.

ratio = 2.9 ± 0.41 , $P < 0.05$).

In spontaneously beating right atria, rec huTNF pretreatment did not affect either the baseline rate of beating or the isoprenaline-induced tachycardia at any concentration.

2 Effect of systemic (i.v.) administration of rec huTNF on rat isolated atria

When rec huTNF was given to rats 1 h before they were killed, the inotropic response of left atria to isoprenaline administration was markedly attenuated; the isoprenaline CRC was shifted to the right accompanied by a decrease in the maximum response (0.475 ± 0.1 g vs 0.765 ± 0.15 g in rec huTNF compared to saline-treated rats, $P < 0.05$; Figure 2a). When the atria were removed 24 h after rec huTNF administration, the suppression of the isoprenaline CRC was no greater than that seen in those removed at 1 h (Figure 2b). Again at neither time point did rec huTNF modify the chronotropic effects of isoprenaline in spontaneously beating right atria.

3 Effects of rec huTNF pretreatment on rat isolated aortic rings—interaction with the L-arginine-NO pathway

Phenylephrine caused a concentration-dependent increase in developed tension in all aortic preparations used.

Tissues removed at 1 h In tissues that had been denuded, the phenylephrine CRC was always to the left of those that had

intact endothelium ($P < 0.05$; compare Figures 3a and 3b). This was true for tissues from both saline and rec huTNF-treated rats. However, there was no difference in the response to phenylephrine in either denuded or endothelium intact preparations from saline or rec huTNF-treated rats.

When tissues were constricted to 70% of maximum with phenylephrine, a vasodilator response to L-arginine could be demonstrated only in rings with an intact endothelium. This vasodilatation was similar in rings from both saline and rec huTNF-treated rats (Figure 4a). D-Arginine was without effect in any of the tissues studied.

When phenylephrine was given at a concentration that would normally elicit a 70% constriction, in the additional presence of L-NAME, the constrictor response was enhanced. This occurred only in rings with an intact endothelium. Furthermore, this potentiation was similar in tissues from saline ($+78 \pm 11\%$) and rec huTNF-treated ($+81 \pm 11\%$) rats (Figure 4b). No augmentation of the constrictor response to phenylephrine was seen in denuded tissues from either group of rats.

Tissues removed at 24 h In saline-treated rats, the relative potency of phenylephrine was again less in endothelium-intact tissues than in denuded tissues. However, in rings from rec huTNF-treated rats this difference was much more marked; the constrictor response to phenylephrine being attenuated both in terms of the EC_{50} of the CRC and the maximum response in intact compared to denuded tissues (compare Figures 5a and b). Hence, although there were no significant

Figure 3 Effect of 1 h saline (1 ml kg^{-1} , i.v., ○) or recombinant human tumour necrosis factor (rec huTNF) ($1 \mu\text{g kg}^{-1}$, i.v., ●) pretreatment on the phenylephrine-induced constrictor response in rat isolated aortic rings. Tissues were either denuded of endothelium (a) or had the endothelium intact (b). Values are means with s.e.mean shown by vertical lines; $n = 6$ for each group.

differences in the phenylephrine responses between endothelium-denuded tissues from saline and rec huTNF-treated rats, the response in endothelium intact tissues from rec huTNF-treated rats was significantly ($P < 0.05$) attenuated. In the presence of phenylephrine (giving a 70% maximal constriction), L-arginine caused a concentration-dependent decrease in developed tension in endothelium intact tissues only. This vasodilatation was significantly greater in rings from rec huTNF-treated ($81 \pm 3\%$) than from saline-treated rats ($41 \pm 2\%$, $P < 0.05$; Figure 6a); D-Arg was without effect.

Furthermore, with the same concentration of phenylephrine, prior administration of L-NAME resulted in an enhanced constrictor response that was significantly greater in tissues from rec huTNF-treated ($+137 \pm 29\%$) than from saline-treated ($+66 \pm 14\%$, $P < 0.05$; Figure 6b). Moreover, under these conditions, an enhanced constrictor response to phenylephrine could also be demonstrated in denuded tissues from rec huTNF-treated rats but not from saline-treated rats.

Discussion

Many types of cardiovascular shock, such as haemorrhagic or septic shock, in man and in animals (Lefer, 1970; 1974) are associated with a depression of cardiac contractility. Given that many proteins and peptides, particularly cytokines such as TNF, interleukin-1 (IL-1), IL-6 and IL-8, are released into the plasma during shock (Martich *et al.*, 1991)

Figure 4 (a) Effect of L-arginine administration on phenylephrine precontracted aortic rings (endothelium intact) from rats given either saline (1 ml kg^{-1} , i.v., ○) or recombinant human tumour necrosis factor- α (rec huTNF), ($1 \mu\text{g kg}^{-1}$, i.v., ●) 60 min beforehand. (b) Percentage increase in developed tension following N^G -nitro-L-arginine methyl ester (L-NAME) treatment in rat aortic rings precontracted to 70% maximum with phenylephrine. Tissues were removed 60 min after saline (Sal, 1 ml kg^{-1} , i.v.) or rec huTNF ($1 \mu\text{g kg}^{-1}$, i.v.) administration. Values are means with s.e.mean shown by vertical bars; $n = 6$ for each group.

then any of these agents could potentially affect cardiac contractility. Since TNF is a primary cytokine released during shock (Tracey *et al.*, 1986), can cause cardiac injury in rats (Lefer *et al.*, 1990) and is elevated in the plasma of patients with heart failure (Levine *et al.*, 1990) its cardio-depressive potential was investigated in rats.

In isolated, electrically stimulated left atria, rec huTNF antagonized the isoprenaline-induced inotropic response when administered directly to the organ bath; however, it was unable to reduce the basal contractility of the tissues. Although the rightward shift of the isoprenaline CRC was not large, these data clearly indicate that even with an incubation time as short as 20 min, TNF can modulate cardiac contractility to exogenous agents. Such a rapid onset of action has been recently reported by Kumar *et al.* (1991) who showed that rec huTNF administered directly to isolated myocytes can concentration-dependently reduce cell shortening following only 15 min incubation. In addition, in isolated whole heart preparations, myocardial dysfunction is seen only 30 min after administration of LPS (Bardakhchian & Palchikova, 1987). To investigate the temporal effect of rec huTNF on atrial contractility, rec huTNF was administered systemically to rats which were killed either 1 h or 24 h later and the atria removed. Again at neither time point was there a depression of the resting systolic preload but there were similar marked inhibitions of the isoprenaline CRCs at both

Figure 5 Effect of 24 h saline (1 ml kg^{-1} , i.v., ○) or recombinant human tumour necrosis factor- α (rec huTNF, $1 \mu\text{g kg}^{-1}$, i.v., ●) pretreatment on the phenylephrine-induced constrictor response in rat isolated aortic rings. Tissues were either denuded of endothelium (a) or had the endothelium intact (b). Values are means with s.e.mean shown by vertical lines; $n = 6$ for each group.

times. The inhibition seen at 1 h confirms the observation on atrial contractility seen following direct rec huTNF administration to the organ bath. Since many of the physiological and metabolic effects of TNF involve either enzyme induction or the modulation of gene expression (Aoki *et al.*, 1989; Evans *et al.*, 1989), then a greater effect of rec huTNF at 24 h may have been predicted. However, no such greater effect was apparent in this study.

Although the present study reveals little about the possible mechanism of action of rec huTNF in the depression of the β -adrenoceptor-mediated inotropic response, this cytokine was unable to modify the isoprenaline-mediated chronotropic response. However, it is clear that the effects of rec huTNF on atrial function seen here are mediated at a level of the transduction pathway distal to adenylate cyclase since similar results to those seen with rec huTNF were obtained when IBMX was used as the inotropic/chronotropic agent. Taken together, these data shown that rec huTNF is capable of depressing an agonist-induced inotropic response in rat isolated atrial tissue. Hence, this cytokine may be added to the list of other putative myocardial depressive factors that may cause cardiodepression during cardiovascular shock.

In addition to eliciting cardiodepressive effects, endotoxin-induced shock or TNF administration also results in marked hypotension (Schiller *et al.*, 1991; Wright *et al.*, 1992) and reduced sensitivity to vasopressor agents (Fink *et al.*, 1985). Many recent studies have provided evidence that this decreased vascular sensitivity seen in LPS-treated animals (Gray *et al.*, 1991; Wright *et al.*, 1992) may be related, at

Figure 6 (a) Effect of L-arginine administration on phenylephrine precontracted aortic rings (endothelium intact) from rats given either saline (1 ml kg^{-1} , i.v., ○) or recombinant human tumour necrosis factor- α (rec huTNF, $1 \mu\text{g kg}^{-1}$, i.v., ●) 24 h beforehand. (b) Percentage increase in developed tension following L-NAME treatment in rat aortic rings precontracted to 70% maximum with phenylephrine. Tissues were removed 24 h after saline (1 ml kg^{-1} , i.v.) or rec huTNF ($1 \mu\text{g kg}^{-1}$, i.v.) administration. Values are means with s.e.mean shown by vertical lines; $n = 6$ for each group.

least in part, to a hyperstimulation of the L-arginine-NO pathway. TNF is known to be released from isolated aortic preparations (McKenna, 1990), macrophages (Jorens *et al.*, 1991) and other cell types, including smooth muscle cells (Busse & Mulsch, 1990) following LPS stimulation and can induce the expression of the non-constitutive form of NO-synthase from these cell types (Drapier *et al.*, 1988; Marsden & Ballerman, 1990) and hence, may itself function as a vasodepressor effector molecule.

In aortae taken from rats treated with rec huTNF 1 h before, neither the vasoconstrictor effect of phenylephrine, nor the responses to L-arginine or L-NAME were modified by rec huTNF pretreatment. This was independent of the presence or absence of endothelium. Hence, unlike the isolated atria experiments described above, rec huTNF seems to have no short term depressive effect on rat vascular tissue. However, these data are somewhat at odds with observations made with LPS or TNF *in vivo* where these agents caused an attenuation of noradrenaline-induced pressor responses associated with NO-synthase activation within 30 min of administration (Kilbourn *et al.*, 1990; Gray *et al.*, 1991). It is important to note that in all of the preparations that had an intact endothelium, this vascular layer remained functional, as reflected by the higher EC_{50} values following phenylephrine administration and the marked responses to L-arginine and L-NAME compared to the denuded preparations.

In contrast, in the tissues removed at 24 h, there was a

clear modulation of the vascular response in rats pretreated with rec huTNF. There was a lower vasoconstrictor response to phenylephrine in tissues with the endothelium intact from rats given rec huTNF. However, this suppression was not apparent in tissues that were devoid of endothelium. Although these data on their own do not indicate the involvement of the L-arginine-NO pathway in these responses, there was also clearly a greater vasodilator effect of L-arginine administration in the endothelium-intact rings from the rec huTNF-treated than in the saline-treated rats. Since no effect of L-arginine was seen in denuded tissues from either group of rats, these data could be interpreted to show a vasodepressor effect of rec huTNF mediated through the induction of a form of NO-synthase present in the endothelial layer only of rat aorta. However, this was not the case, since in the additional presence of L-NAME, an enhanced vasoconstrictor response to subsequent phenylephrine administration was seen in rings from rec huTNF-treated rats, not only when the endothelium was present, but also when it was absent. This observation indicates that rec huTNF can

induce a form of NO-synthase in both the endothelial layer and within the smooth muscle layers of the aortic tissue; the latter only being revealed following NO-synthase inhibition. The lack of a vasodilator effect as a result of L-arginine administration to denuded tissues from rec huTNF-treated rats may reflect different mechanisms of L-arginine transport and utilisation in the two layers of the tissue.

Hence, a vasodepressor effect of rec huTNF on phenylephrine-induced vasoconstriction, possibly effected through L-arginine-NO-mediated events, as well as a cardiodepressive action can be demonstrated in isolated tissues from rats. Indeed these effects seen with rec huTNF may be indicative of some of the cardiovascular manifestations (e.g. hypotension, cardiodepression) associated with shock-like states such as septic shock where TNF is thought to be a primary mediator (Tracey *et al.*, 1986). Furthermore, inhibition of TNF action by, for example, anti-TNF monoclonal antibodies, may provide an additional approach to the therapy of patients with profound cardiovascular shock.

References

- AOKI, N., SEIGFRIED, M. & LEFER, A.M. (1989). Anti-EDRF effect of tumor necrosis factor in isolated, perfused cat carotid arteries. *Am. J. Physiol.*, **256**, H1509–H1512.
- BARDAKHCHIAN, E.A. & PALCHIKOVA, E.I. (1987). Ultrastructural evidences of direct endotoxin action on the heart. *Cor Vasa*, **29**, 64–69.
- BEUTLER, B.D. & CERAMI, A. (1987). Cachectin: more than a tumor necrosis factor. *N. Engl. J. Med.*, **316**, 379–385.
- BEUTLER, B.D., GREENWALD, J.D., HULMES, M., CHANG, Y., PAN, C.E., MATHISON, J., ULEVITCH, R. & CERAMI, A. (1985). Identity of tumor necrosis factor and the macrophage secreted factor cachectin. *Nature*, **316**, 552–554.
- BUSSE, R. & MÜLSCH, A. (1990). Induction of nitric oxide synthase by cytokines in vascular smooth muscle cells. *FEBS*, **275**, 87–90.
- DRAPIER, J., WIETXERBIN, J. & HIBBS, J.B. (1988). Interferon gamma and tumor necrosis factor induce the L-arginine-dependent cytotoxic effector mechanism in murine macrophages. *Eur. J. Immunol.*, **18**, 1587–1591.
- EVANS, R.D., ARGILES, J.M. & WILLIAMSON, D.M. (1989). Metabolic effects of tumour necrosis factor (cachectin) and interleukin-1. *Clin. Sci.*, **77**, 357–364.
- FINK, M.P., HOMER, L.D. & FLETCHER, J.R. (1985). Diminished pressor response to exogenous norepinephrine and angiotensin II in septic, non anesthetized rats: evidence for a prostaglandin-mediated effect. *J. Surg. Res.*, **38**, 335–342.
- FLEMING, I., JOLOU-SCHAEFFER, G., GRAY, G.A., PARRATT, J.R. & STOCLET, J.-C. (1991). Evidence that an L-arginine/nitric oxide dependent elevation of tissue cyclic GMP content is involved in depression of vascular reactivity by endotoxin. *Br. J. Pharmacol.*, **103**, 1047–1052.
- GOODYEAR, A.V.N. (1967). Left ventricular function and tissue hypoxia in irreversible hemorrhagic and endotoxin shock. *Am. J. Physiol.*, **212**, 44.
- GRAY, G.A., SCOTT, C., JOLOU-SCHAEFFER, G., FLEMING, I., PARRATT, J.R. & STOCLET, J.-C. (1991). The effect of inhibitors of L-arginine/nitric oxide pathway on endotoxin-induced loss of vascular responsiveness in anaesthetized rats. *Br. J. Pharmacol.*, **103**, 1218–1224.
- HINSHAW, L.B., TEKAMP-OLSEN, P., CHANG, A.C.K., LEE, A.P., TAYLOR, F.B., MURRAY, C.K., PEER, G.T., EMERSON, T.E., PASSEY, R.B. & KUO, G.C. (1990). Survival of primates in LD₁₀₀ septic shock following therapy with antibody to tumor necrosis factor (TNF). *Circ. Shock*, **30**, 279–292.
- JORENS, P.G., VAN OVERVELD, F.J., BULT, H., VERMIERE, P.A. & HERMAN, A.G. (1991). L-arginine-dependent production of nitrogen oxides by rat pulmonary macrophages. *Eur. J. Pharmacol.*, **200**, 205–209.
- KILBOURN, R.G. & BELLONI, P. (1990). Endothelial cell production of nitrogen oxides in response to interferon in combination with tumor necrosis factor, interleukin-1 or endotoxin. *J. Natl. Cancer Inst.*, **82**, 772–776.
- KILBOURN, R.G., JUBRAN, A., GROSS, S.S., GRIFFITH, O.W., LEVI, R., ADAMS, J. & LODATO, R.F. (1990). Reversal of endotoxin-mediated shock by N^G-methyl-L-arginine, an inhibitor of nitric oxide synthesis. *Biochem. Biophys. Res. Commun.*, **172**, 1132–1138.
- KUMAR, A., DIMOU, C., HOLLENBERG, S.M., CUNNION, R.E., LAWRENCE, M., URETZ, E., SNELL, R.J. & PARRILLO, J.E. (1991). Tumor necrosis factor produces a concentration-dependent depression of myocardial cell contraction *in vitro*. *Clinical Res.*, **39**, 321A.
- LEFER, A.M. (1970). Role of myocardial depressant factor in the pathogenesis of circulatory shock. *Fed. Proc.*, **29**, 1836–1847.
- LEFER, A.M. (1974). Myocardial depressant factor and circulatory shock. *Klin. Wochens.*, **52**, 358–370.
- LEFER, A.M. & ROVETTO, M.J. (1970). Influence of myocardial depressant factor on physiologic properties of cardiac muscle. *Proc. Soc. Exp. Biol. Med.*, **134**, 269–273.
- LEFER, A.M., TSAO, P., AOKI, N. & PALLADINO, M.A. (1990). Mediation of cardioprotection by transforming growth factor- β . *Science*, **249**, 61–64.
- LEVINE, B., KALMAN, J., MAYER, L., FILLIT, H. & PACKER, M. (1990). Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. *N. Engl. J. Med.*, **323**, 236–241.
- MCKENNA, T.M. (1990). Prolonged exposure of rat aorta to low levels of endotoxin *in vitro* results in impaired contractility. *J. Clin. Invest.*, **86**, 160–168.
- MARSDEN, P.A. & BALLERMAN, B.J. (1990). Tumor necrosis factor activates soluble guanylate cyclase in bovine glomerular mesangial cells via an L-arginine-dependent mechanism. *J. Exp. Med.*, **172**, 1843–1852.
- MARTICH, G.D., DANNER, R.L., CESKA, M. & SUFFREDINI, A.F. (1991). Detection of interleukin 8 and tumor necrosis factor in normal humans after intravenous endotoxin: the effect of antiinflammatory agents. *J. Exp. Med.*, **173**, 1021–1024.
- MATHISON, J.C., WOLFSON, E. & ULEVITCH, R.J. (1988). Participation of tumor necrosis factor in the mediation of gram negative bacterial lipopolysaccharide-induced injury in rabbits. *J. Clin. Invest.*, **81**, 1925–1937.
- SCHILLER, J.H., STORER, B.E., WITT, P.L., ALBERTI, D., TOMBES, M.B., ARZOOMANIAN, R., PROCTOR, R.A., MCCARTHY, D., BROWN, R.R., VOSS, S.D., REMICK, S.C., GREM, J.L., BORDEN, E.C. & TRUMP, D.L. (1991). Biological and clinical effects of tumor necrosis factor- α administered three times weekly. *Cancer Res.*, **51**, 1651–1658.
- TRACEY, K.J., FONG, Y., HESSE, D.J., MANOGUE, K.R., LEE, A.T., KUO, G.C., LOWRY, S.F. & CERAMI, A. (1987). Anti-cachectin/TNF antibodies protect septic shock during lethal bacteremia. *Nature*, **330**, 662–664.
- WRIGHT, C.E., REES, D.D. & MONCADA, S. (1992). Protective and pathological roles of nitric oxide in endotoxin shock. *Cardiovasc. Res.*, **26**, 48–57.

(Received March 6, 1992

Revised April 3, 1992

Accepted April 10, 1992)