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Lack of effect of TNF antibodies on the cardiovascular sequelae of lipopolysaccharide infusion in conscious rats

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1 The aims of the present study were to determine the profile of tumour necrosis factor (TNF) release, and the effect of monoclonal antibodies to TNF, on the changes in regional haemodynamics and the responses to vasodilator and vasoconstrictor challenges, during a continuous 24 h low dose infusion of lipopolysaccharide (LPS) in conscious rats.

2 Male Long Evans rats were chronically instrumented for measurement of regional haemodynamics (renal, superior mesenteric and hindquarters) and were challenged with 3 min infusions of acetylcholine (22 nmol min^{-1}), methoxamine (120 nmol min^{-1}), salbutamol (0.83 nmol min^{-1}) and bradykinin (14.4 nmol min^{-1}). The rats were given either saline, or the TNF antibodies, TN3g1 or TN3g2a, 1 h before the start of a continuous infusion of LPS (150 $\mu g\ kg^{-1}\ h^{-1}$) and were subsequently re-tested with the vasodilator and vasoconstrictor challenges 2, 6 and 24 h after the start of the LPS infusion.

3 Prior to infusion of LPS, TNF was not detectable in the plasma. During continuous infusion of LPS there was a transient elevation in plasma TNF levels, reaching a maximum ($\sim$2300 pg ml^{-1}) after approximately 1 h, and returning to undetectable levels after approximately 3 h of LPS infusion.

4 In the saline pretreated group, after 1-2 h of LPS infusion, there was a small hypotension and a marked renal vasodilatation; 6 h after the start of LPS infusion there was a minor elevation in MAP above control levels, renal vasodilatation was maintained and a hindquarters vasoconstriction occurred; after 24 h of LPS infusion, there was a hypotension and renal and hindquarters vasodilatation. There were significant reductions in the tachycardic and renal vasodilator responses and an enhanced depressor response to acetylcholine after 24 h of LPS infusion. LPS infusion also caused a generalized hyporesponsiveness to the cardiovascular effects of methoxamine and salbutamol. The major changes in response to bradykinin were reduced tachycardic and enhanced depressor responses throughout the LPS infusion, a biphasic decrease and increase in renal conductance and enhanced hindquarters vasodilatation at 24 h.

5 Pretreatment with either TN3g1 or TN3g2a antibodies had no major effects on the changes in resting haemodynamics, or on the changes in response to methoxamine, salbutamol or bradykinin challenges during LPS infusion. However, the tachycardic responses to acetylcholine were generally preserved, and its hypotensive effect after 24 h of LPS infusion was not enhanced after TN3g1 or TN3g2a pretreatment. Thus, despite substantial, but transient, elevation of plasma TNF levels during continuous LPS infusion, it appears that the majority of cardiovascular changes were dependent on factors other than plasma TNF.

Keywords: TNF antibody; regional haemodynamics; lipopolysaccharide; conscious rat

Introduction

Septic shock is characterized by severe cardiovascular collapse with hypotension, organ failure and ultimately death. In the case of Gram-negative bacteraemia, the primary initiator of this syndrome is thought to be lipopolysaccharide (LPS), which activates host cells to produce numerous endogenous mediators, including cytokines such as interleukins (IL), interferons (IFN) and tumour necrosis factor (TNF) (see Billiau & Vandekerckhove, 1991, for review).

Evidence for the involvement of TNF in sepsis comes from reports of elevated plasma levels of TNF in patients with septic shock, and the fact that LPS administration can increase plasma levels of TNF in healthy human subjects and animals (see Van der Poll & Lowry, 1995 for review). In addition, several studies have shown that administration of TNF to animals results in the development of many of the characteristics of septic shock, including hypotension, metabolic acidosis and organ damage (Tracey *et al.*, 1986; 1987b; Remick *et al.*, 1987; Schirmer *et al.*, 1989). However, other studies have indicated that TNF is not capable of initiating the characteristics of septic shock in animals in the absence of LPS. Indeed, Rothstein & Schreiber, (1988) demonstrated that non-lethal doses of LPS could cause death in mice when given in combination with a non-lethal dose of TNF, suggesting a synergistic interaction. Similarly, work carried out in the rat demonstrated that TNF could cause metabolic acidosis, hypotension and death only when given with a sublethal dose of LPS (Neilson *et al.*, 1989). The latter authors suggested that the reports demonstrating the lethality of TNF when given alone, could possibly be explained by endotoxin contamination of the TNF source, or pre-existing endotoxaemia in the animals studied.

Perhaps the most convincing evidence for the involvement of TNF in animal models of endotoxaemia comes from studies demonstrating that passive immunisation with TNF antibodies reduces mortality (Beutler *et al.*, 1985a; Tracey *et al.*, 1987a; Mathison *et al.*, 1988; Hinshaw *et al.*, 1990; Suitters *et al.*, 1994), and attenuates certain cardiovascular sequelae (Tracey *et al.*, 1987a; Mathison *et al.*, 1988; Hinshaw *et al.*, 1990; Jesmok *et al.*, 1992; Thiemermann *et al.*, 1993).

Studies in mice have demonstrated that changing the isotype (Fc region) of a hamster anti-murine TNF monoclonal antibody (TN3-19.12), previously shown to neutralize TNF *in vivo* (Sheehan *et al.*, 1989), alters the ability of the antibody to

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prevent LPS-induced mortality (Suitters *et al.*, 1994). Thus, substitution of the hamster Fc region with a murine g1 (TN3g1) Fc region produced an antibody which was more effective at preventing mortality in mice (10% mortality after 72 h) after a lethal injection of LPS (100% mortality after 27 h), than substitution with a murine g2a (TN3g2a) Fc region (90% mortality after 72 h). The differences in the protective ability of the antibodies are thought to relate to the different properties of the Fc regions. The antibody containing the g1 Fc region forms a simple antigen-antibody complex, neutralizing, and hence preventing, the harmful actions of TNF. However, the antibody containing the g2a Fc region can activate both the complement cascade and Fc receptors on immune cells leading to increased activation of the inflammatory system with subsequent detrimental effects.

We have previously described the regional and cardiac haemodynamic responses to administration of a continuous low dose infusion of LPS in conscious rats (Gardiner *et al.*, 1992a; 1994; Waller *et al.*, 1994). In addition, we also described changes in responses to acetylcholine, methoxamine, salbutamol and bradykinin during infusion of LPS (Waller *et al.*, 1994). These substances were chosen to allow assessment of a wide range of mechanisms all of which have been reported to be affected by endotoxaemia. Thus, haemodynamic responses to acetylcholine, salbutamol and bradykinin all contain components sensitive to inhibition of nitric oxide synthase (NOS) (Gardiner *et al.*, 1990d; 1991b). In addition, β_2-adrenoceptors are involved in the responses to bradykinin (Gardiner *et al.*, 1992b), but bradykinin also elicits responses insensitive to NOS inhibition, or β_2-adrenoceptor antagonism (Gardiner *et al.*, 1990d; 1992b). Methoxamine was used to assess the reported development of hyporesponsiveness to α-adrenoceptor agonists in endotoxaemia (e.g. Chernow *et al.*, 1982; Guc *et al.*, 1990).

The aims of the present study were, therefore, to determine the plasma TNF profile, and to compare the effects of TN3g1 and TN3g2a pretreatment with saline pretreatment, on the changes in regional haemodynamics and responses to vasoactive agents, during a continuous low dose infusion of LPS in the conscious rat.

Methods

Regional haemodynamics

All experiments were carried out in male Long Evans rats (350–450g). Animals were anaesthetized (sodium methohexitone, 60 mg kg^{-1}, i.p., supplemented as required) and miniaturized pulsed Doppler probes (Haywood *et al.*, 1981) were implanted around the left renal and superior mesenteric arteries and the distal aorta below the level of the ileocaecal artery (to monitor flow to the hindquarters) (Gardiner *et al.*, 1990b,c,d). Probe wires were led subcutaneously to the back of the neck and secured. After 7–14 days in their home cages, the rats were anaesthetized (sodium methohexitone, 40–60 mg kg^{-1}, i.p., supplemented as required) and implanted with i.v. catheters in the jugular vein for drug administration, and an i.a. catheter in the distal abdominal aorta (via the caudal artery) for measurement of mean arterial blood pressure and heart rate. The catheters were then taken subcutaneously to the back of the neck. Rats were fitted with a small harness to which a spring was attached (through which the catheters ran) leading to a counterbalanced arm. Finally, a custom-built probe wire connector was attached to the spring which allowed all subsequent procedures to be performed remote from the animals. Double channel swivels (Blair *et al.*, 1980) were used to allow overnight i.v. infusion of saline or LPS, and also i.a. infusion of heparinized saline (15 u ml^{-1}) to maintain catheter patency. During the experiment, continuous recordings were made of heart rate, mean and phasic blood pressure and mean and phasic renal, mesenteric and hindquarters Doppler shift signals (using a modified (Gardiner *et al.*, 1990a) pulsed Doppler system (Crystal Biotech, Holliston, U.S.A.)). Changes in regional vascular conductances were calculated from mean Doppler shift and MAP (Gardiner *et al.*, 1990b,c,d).

Following a 30 min period of recording baseline cardiovascular variables, rats were challenged with 3 min infusions (0.15 ml min^{-1}) of acetylcholine (22 nmol min^{-1}), methoxamine (120 nmol min^{-1}), salbutamol (0.83 nmol min^{-1}) and bradykinin (14.4 nmol min^{-1}), in random order, with at least 10 min between each infusion. The animals then received an infusion (over 10 min) of either saline (2.5 ml; $n=8$), TN3g1 (20 mg kg^{-1} in 1.59 ml; $n=8$) or TN3g2a (20 mg kg^{-1} in 2.5 ml; $n=8$) followed 1 h later by a continuous, 24 h infusion (0.3 ml h^{-1}) of LPS (150 μg kg^{-1} h^{-1}) in saline. Rats were then challenged with acetylcholine, methoxamine, salbutamol and bradykinin after 2, 6 and 24 h of LPS infusion. The experimental design meant that it was not possible to administer more than one dose of each of the vasoactive substances. The single doses were therefore chosen on the basis of our previous work (Gardiner *et al.*, 1991b; 1992b; Waller *et al.*, 1994). The doses of antibodies used in the present study were chosen on the basis of previous work (Suitters *et al.*, 1994), and pilot experiments in which the appearance of detectable TNF in the plasma in our model of endotoxaemia was completely prevented (data not shown).

Plasma TNF levels

Male Long Evans rats (350–450g) were anaesthetized (60 mg kg^{-1}, i.p. supplemented as required) and implanted with i.v. (for LPS administration) and i.a. (for blood sampling) catheters, as described above. Blood samples (~0.6 ml) were obtained before and after the start of LPS infusion over 24 h (with at least 2 h between each sample; maximum of 5 samples per rat). Samples were then centrifuged immediately, the plasma extracted and each sample was frozen (−80°C) until an ELISA was performed for TNF detection (Hewitt *et al.*, 1995). Briefly, samples were incubated in microtitre plates coated with TN3 19.12 and captured rat TNFα was revealed with a rabbit polyclonal raised against mouse TNF which cross-reacted with rat TNF (Endogen). Bound antibody was revealed with horseradish peroxidase conjugated donkey anti-rabbit IgG (Jacksons) followed by TMB substrate.

Data analysis

Intra-group changes relative to the baseline, and changes in responses to vasoactive substances based on the areas under or over the curves (AUC or AOC during 3 min drug infusions), were analysed by Friedman's test (Theodorsson-Norheim, 1987) or Wilcoxon's signed rank test as appropriate. Intergroup changes were analysed by the Kruskal-Wallis test; a P value <0.05 was taken to indicate a significant difference.

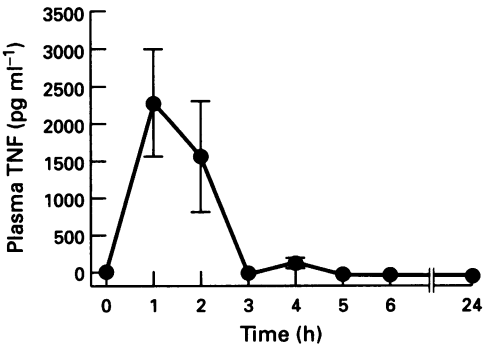

Figure 1 Plasma TNF levels during a 24 h lipopolysaccharide infusion (150 μg kg^{-1} h^{-1}) in conscious Long Evans rats ($n \geq 6$ for each time point). Values are means ± s.e.mean. Values of zero represent plasma TNF levels below the detection level of the assay used.

Drugs

Acetylcholine chloride (Sigma), methoxamine hydrochloride B.P. (Wellcome Foundation), and lipopolysaccharide (*E. coli* serotype 0127:B8)(Sigma) were dissolved in isotonic saline. Bradykinin (Bachem, U.K.), was dissolved in saline containing 1% bovine serum albumin, and salbutamol hemisulphate salt (Sigma) was dissolved in saline containing ascorbic acid (1 mmol l^{-1}). The hamster murine chimeric anti-TNF monoclonal antibodies (TN3g1 and TN3g2a, Suitters *et al.*, 1994) (Celltech Therapeutics) were obtained in sterile antibody solution (TN3g1:- 50 mM sodium acetate, 100 mM NaCl; TN3g2a:- 50 mM sodium acetate) at pH 5.5.

Results

Plasma TNF profile during LPS infusion

Prior to infusion of LPS, TNF was not detectable in plasma (Figure 1). Plasma levels of TNF reached a maximum (~2300 pg ml^{-1}) approximately 1 h after the start of LPS infusion, and were again undetectable after approximately 3 h of LPS infusion (Figure 1).

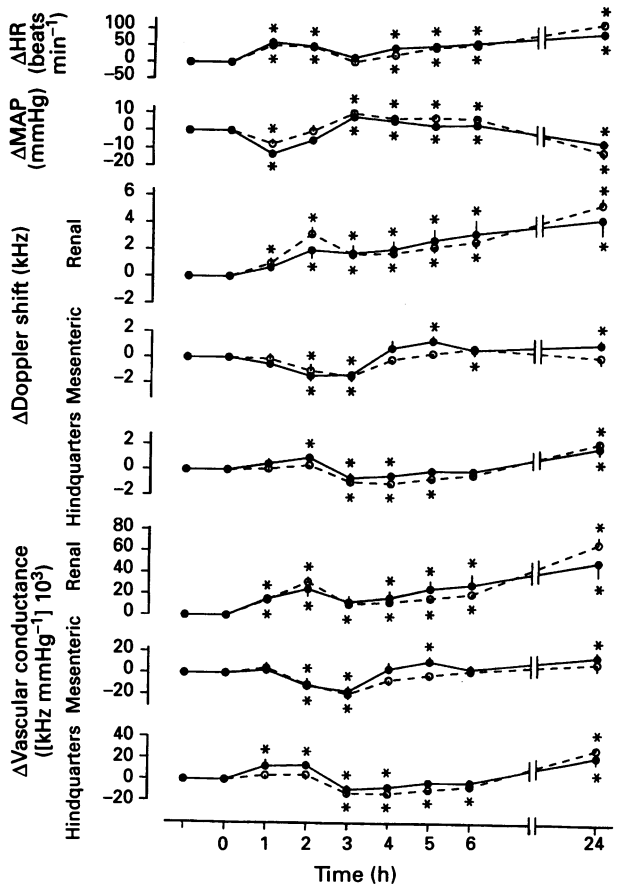

Figure 2 Changes in resting heart rate (HR), mean arterial pressure (MAP) and renal, mesenteric and hindquarters Doppler shift and vascular conductance during a 24 h lipopolysaccharide infusion (150 $\mu g\,kg^{-1}\,h^{-1}$) after saline (○) or TN3g1 (●) pretreatment in conscious Long Evans rats ($n=8$ in each group). Values are mean with s.e.mean, $^*P<0.05$ versus time 0 (Friedman's test).

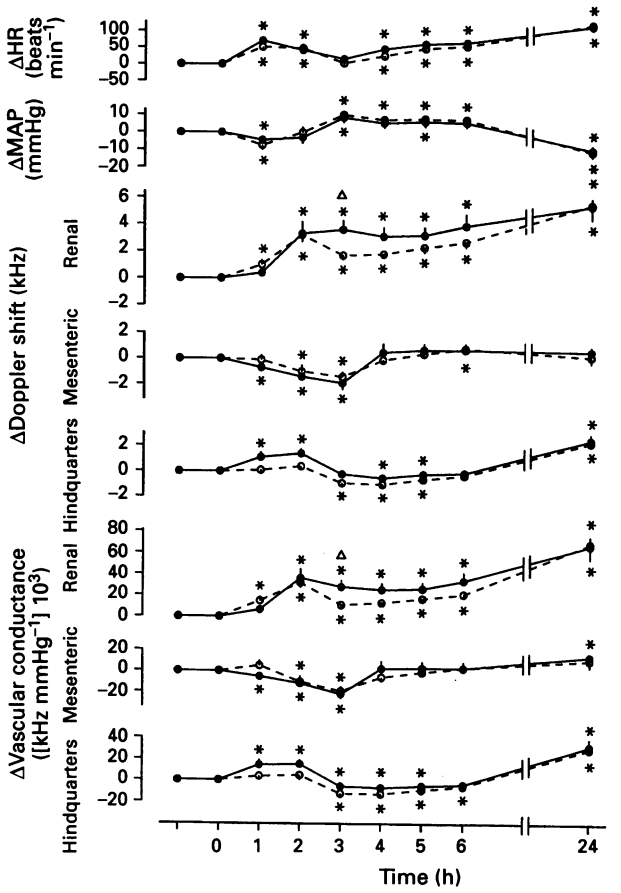

Figure 3 Changes in resting heart rate (HR), mean arterial pressure (MAP) and renal, mesenteric and hindquarters Doppler shift and vascular conductance during a 24 h lipopolysaccharide infusion (150 $\mu g\,kg^{-1}\,h^{-1}$) after saline (○) or TN3g2a (●) pretreatment in conscious Long Evans rats ($n=8$ in each group). Values are mean with s.e.mean, $^*P<0.05$ versus time 0 (Friedman's test); Δ $P<0.05$ versus saline pretreated group (Kruskal-Wallis test).

Table 1 Resting cardiovascular variables before and 1 h after saline, TN3gl (20 mg kg^{-1}) or TN3g2a (20 mg kg^{-1}) administration

	Saline		*TN3gl*		*TN3g2a*	
	Control	*1 h*	*Control*	*1 h*	*Control*	*1 h*
Heart rate (beats min^{-1})	351±4	342±5	335±7	331±8	344±5	344±7
Mean arterial pressure (mmHg)	102±2	99±2	102±2	98±2	105±2	99±3
Renal flow (kHz)	5.6±0.4	5.5±0.4	5.1±0.3	4.9±0.3	5.9±1.1	5.6±0.8
Mesenteric flow (kHz)	8.2±1.0	6.9±0.7*	6.7±0.7	5.7±0.7*	6.7±0.6	6.2±0.7*
Hindquarters flow (kHz)	3.9±0.2	3.7±0.2	4.7±0.6	4.2±0.6	5.1±0.6	4.6±0.5
Renal conductance ([kHz $mmHg^{-1}$] 10^3)	55±3	56±4	49±3	50±3	56±10	56±8
Mesenteric conductance ([kHz $mmHg^{-1}$] 10^3)	80±9	70±8*	66±7	58±7*	64±5	62±6
Hindquarters conductance ([kHz $mmHg^{-1}$] 10^3)	38±3	38±2	46±6	43±6	49±6	47±5

Values means±s.e.mean. $n=8$. $^*P<0.05$ versus resting value.

Resting cardiovascular variables before and during infusion of LPS after pretreatment with saline, or TN3g1, or TN3g2a

Resting cardiovascular variables, both before and 1 h after saline, TN3g1 or TN3g2a pretreatment are shown in Table 1. Over the 1 h following saline or antibody administration there was a significant reduction in mesenteric flow in all three groups, associated with reductions in mesenteric vascular conductance in the saline and TN3g1 pretreated groups; these changes have been described previously (Gardiner *et al.*, 1991a), and are likely to be attributable to the feeding pattern of the rat. During infusion of LPS, the haemodynamic profile was similar to that described previously (Figure 2) (Waller *et al.*, 1994).

The pattern of haemodynamic changes observed during infusion of LPS in the animals pretreated with TN3g1, or TN3g2a, was similar to that seen in the animals pretreated with saline. The only significant difference was observed in the TN3g2a pretreated group, in which renal flow and conductance were significantly higher than in the saline pretreated group after 3 h of LPS infusion (Figure 3).

Responses to acetylcholine before and during LPS infusion after pretreatment with saline, or TN3g1, or TN3g2a

Before pretreatment with saline, TN3g1 or TN3g2a, acetylcholine infusion caused a depressor and tachycardic response associated with substantial increases in renal flow and conductance, small reductions in mesenteric flow and conductance, and a small increase in hindquarters conductance (Figure 4). During LPS infusion in the saline pretreated group, the changes in the responses to acetylcholine (Figure 4) were similar to those reported previously (Waller *et al.*, 1994). The major features of note were inhibition of the tachycardic and renal vasodilator responses at a time when the depressor response was enhanced.

The profile of change in response to acetylcholine challenge after the onset of LPS infusion in both antibody pretreated groups was generally similar to that observed in the saline pretreated group (Figure 4). The only differences observed in the TN3g1 pretreated group, compared to the saline pretreated group, were that the tachycardic response in the former group was not significantly reduced at any time point, the hypoten-

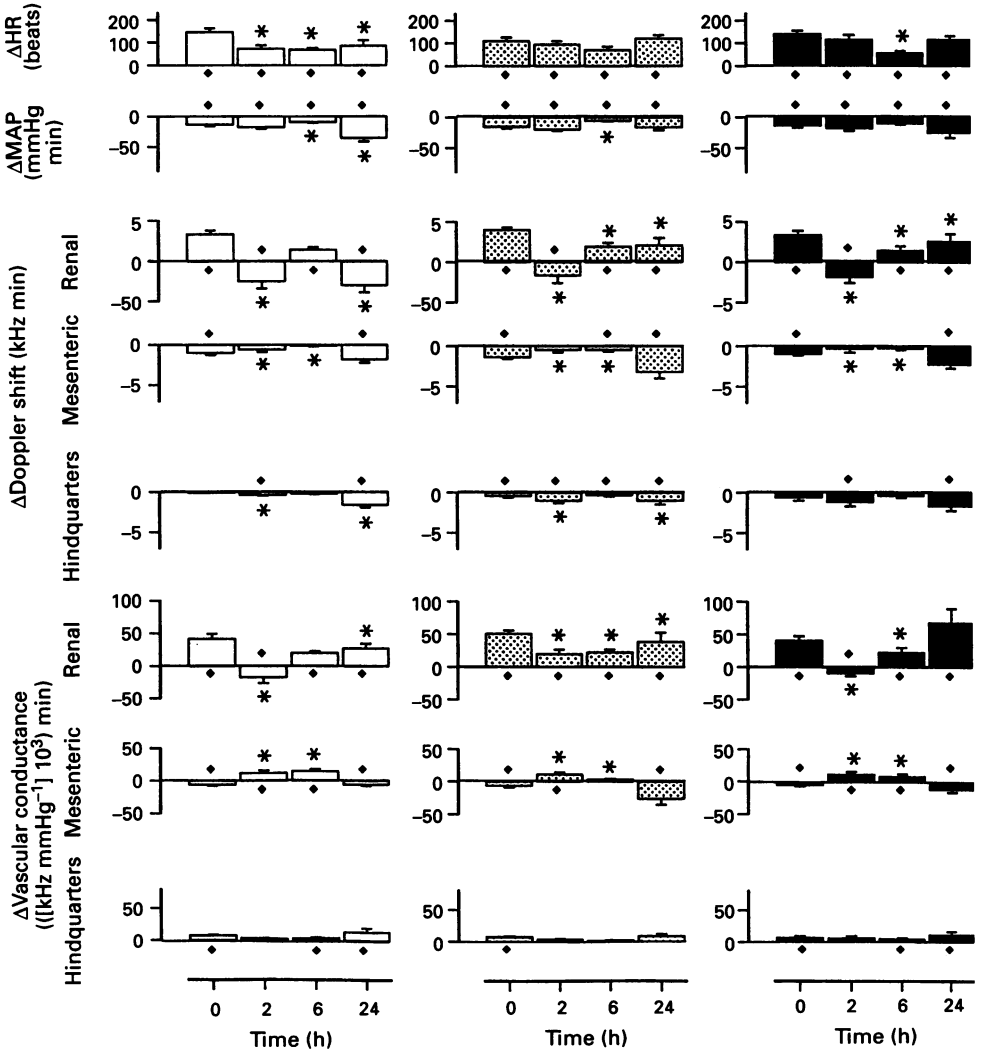

Figure 4 Changes (areas under or over curves, 0–3 min) in heart rate (HR), mean arterial pressure (MAP) and renal, mesenteric and hindquarters Doppler shift and vascular conductance in response to acetylcholine infusion (22 nmol min^{-1}) before and during a 24 h lipopolysaccharide infusion (150 μg kg^{-1} h^{-1}) after saline (open columns), TN3g1 (stippled columns) or TN3g2a (solid columns) pretreatment, in conscious Long Evans rats ($n=8$ in each group). Values are mean with s.e.mean, *$P<0.05$ versus time 0 (Friedman's test); ◆ represents a significant response ($P<0.05$, Wilcoxon's signed rank test).

sive response was not enhanced at 24 h, and there was a small renal vasodilator response at 2 h, rather than the vasoconstrictor response seen in the saline pretreated group (Figure 4). The only differences observed in the TN3g2a pretreated group, compared to the saline pretreated group, were apparent after 24 h of LPS infusion when the tachycardic, hypotensive, and renal vasodilator responses were normal (Figure 4).

Responses to methoxamine before and during LPS infusion after pretreatment with saline, or TN3g1 or TN3g2a

Infusion of methoxamine before pretreatment with saline, TN3g1 or TN3g2a caused a pressor and bradycardic response associated with reductions in mesenteric and hindquarters flows and conductances and a modest increase in renal flow associated with a vasoconstriction (Figure 5). The changes in the responses to methoxamine in the saline pretreated group during LPS infusion (Figure 5) were as described elsewhere (Waller *et al.*, 1994), namely reduced bradycardic, pressor and renal, mesenteric and hindquarters vasoconstrictor responses. Overall, these changes were unaffected by pretreatment with either of the TNF antibodies (Figure 5).

Responses to salbutamol before and during LPS infusion after pretreatment with saline, or TN3g1, or TN3g2a

Prior to administration of saline, TN3g1 or TN3g2a, salbutamol infusion caused a depressor and tachycardic response associated with substantial increases in hindquarters flow and conductance, an increase in renal conductance, and a reduction in mesenteric flow associated with a small mesenteric vasodilatation (Figure 6). During LPS infusion in the saline pretreated group, the changes in the responses to salbutamol (Figure 6) were consistent with our earlier findings (Waller *et al.*, 1994), i.e., attenuated depressor, tachycardic and hindquarters vasodilator effects. This pattern was also seen in the presence of either antibody (Figure 6).

Responses to bradykinin before and during LPS infusion after pretreatment with saline, or TN3g1, or TN3g2a

Before pretreatment with saline, TN3g1 or TN3g2a, bradykinin caused a tachycardic and depressor response, associated with a substantial increase in mesenteric flow and conductance and smaller increases in renal and hindquarters flows and conductances (Figure 7). The changes in the re-

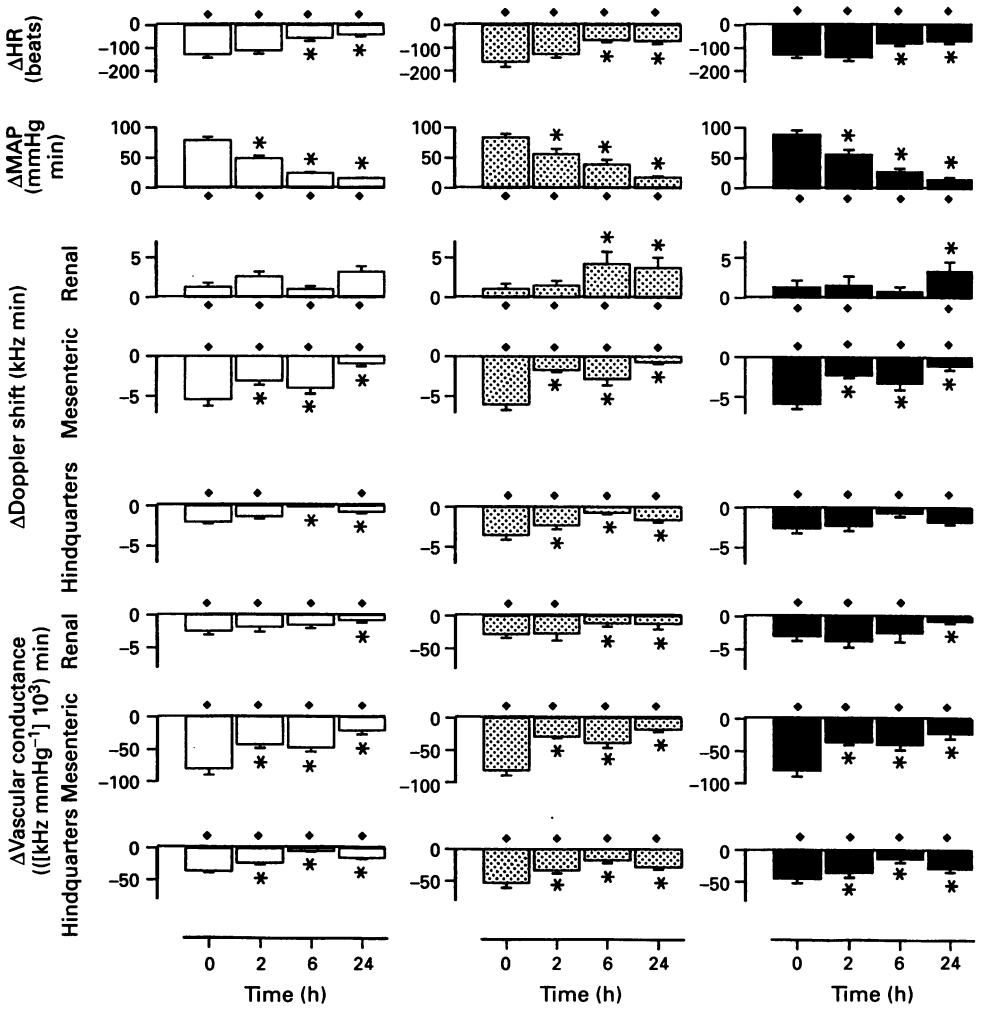

Figure 5 Changes (areas under or over curves 0–3 min) in heart rate (HR), mean arterial pressure (MAP) and renal, mesenteric and hindquarters Doppler shift and vascular conductance in response to methoxamine infusion (120 nmol min^{-1}) before and during a 24 h lipopolysaccharide infusion (150 μg kg^{-1} h^{-1}) after saline (open columns), TN3g1 (stippled columns) or TN3g2a (solid columns) pretreatment, in conscious Long Evans rats ($n=8$ in each group). Values are mean with s.e.mean, *$P<0.05$ versus time 0 (Friedman's test); ◆ represents a significant response ($P<0.05$, Wilcoxon's signed rank test).

sponses to bradykinin in the saline pretreated group during LPS infusion were complex (Figure 7), and similar to those reported previously (Waller *et al.*, 1994), with enhanced depressor and reduced tachycardic responses, associated with renal and hindquarters vascular effects which changed with time.

After the start of LPS infusion in the TN3g1 and TN3g2a pretreated groups, the changes in the responses to bradykinin were similar to those observed in the saline pretreated group (Figure 7).

Discussion

The present study shows that a continuous, low dose infusion of LPS resulted in a rapid (within 1 h) peak in plasma TNF levels, with a return towards baseline after approximately 3 h. Moreover, the LPS-induced changes in resting cardiovascular variables, and changes in response to acetylcholine, methoxamine, salbutamol and bradykinin challenges, seen during LPS infusion, were only very modestly affected by pretreatment with the TNF monoclonal antibodies TN3g1, or TN3g2a.

Previous studies have demonstrated increases in plasma levels of TNF in a variety of animal models of endotoxaemia, e.g., following caecal ligation and puncture (Eskandari *et al.*, 1992), or administration of LPS (Beutler *et al.*, 1985b; Waage, 1987; Mathison *et al.*, 1988), or bacteria (Tracey *et al.*, 1987a; Silva *et al.*, 1990). The profile of plasma TNF changes described in the present study, i.e., a rapid (1 h) elevation in plasma TNF, with levels returning to control values after 3 h, is similar to that described by Waage (1987), who reported that administration of LPS i.v. to conscious rats, as either a bolus (200 $\mu g\ kg^{-1}$), a bolus (200 $\mu g\ kg^{-1}$) followed by a continuous infusion ($\sim 40\ \mu g\ kg^{-1}\ h^{-1}$), or as a continuous infusion ($\sim 360\ \mu g\ kg^{-1}\ h^{-1}$), resulted in the same transient elevation in plasma TNF levels.

Interestingly, the cardiovascular changes in the present study began after about 1 h, but thereafter developed progressively through to 24 h. Thus, there was an apparent dissociation between the change in plasma TNF levels and the magnitude of the cardiovascular changes observed. One possible explanation is that the early elevation in plasma TNF

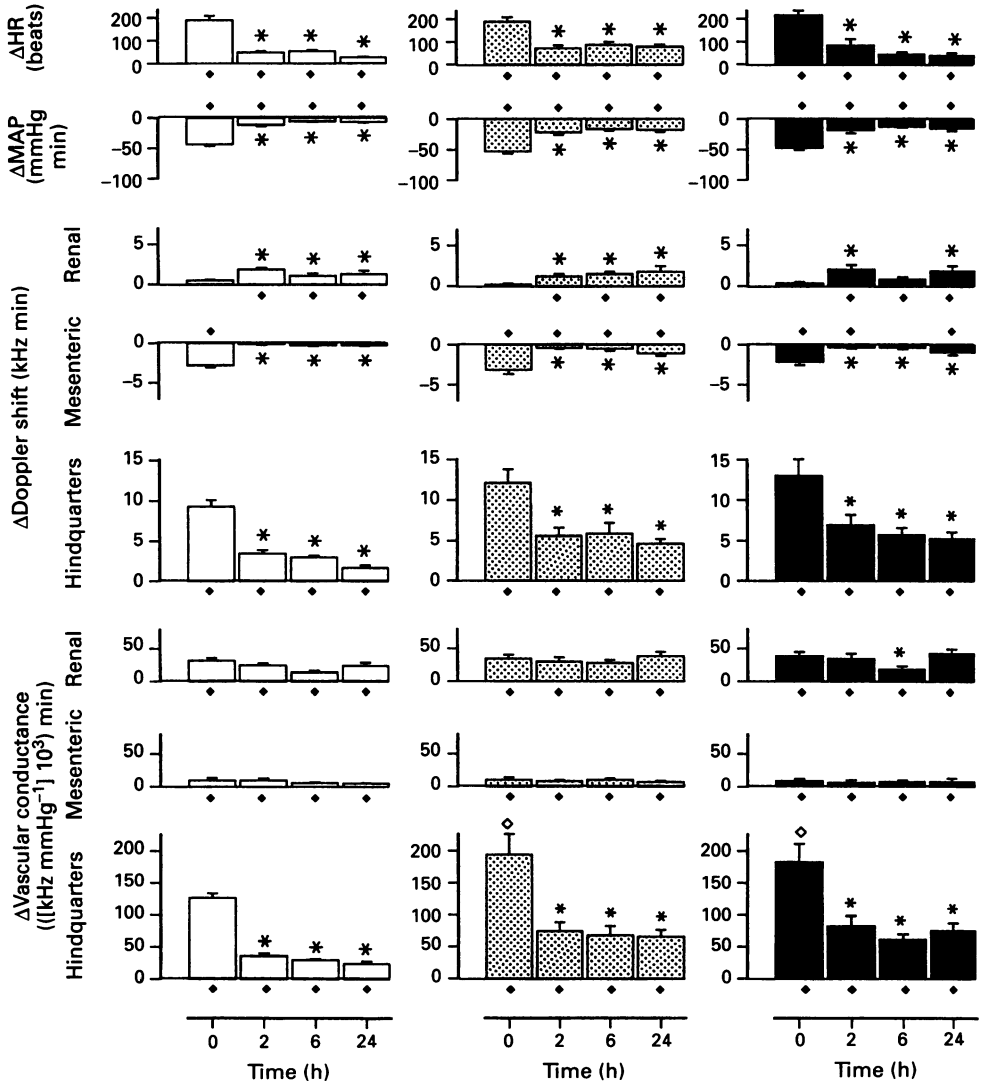

Figure 6 Changes (areas under or over curves 0–3 min) in heart rate (HR), mean arterial pressure (MAP) and renal, mesenteric and hindquarters Doppler shift and vascular conductance in response to salbutamol infusion (0.83 nmol min^{-1}) before and during a 24 h lipopolysaccharide infusion (150 $\mu g\ kg^{-1}\ h^{-1}$) after saline (open columns), TN3g1 (stippled columns), or TN3g2a (solid columns) pretreatment, in conscious Long Evans rats ($n=8$ in each group). Values are mean with s.e.mean, $^*P<0.05$ versus time 0 (Friedman's test); $^{\diamond}$ $P<0.05$ versus control group at time 0 (Kruskal-Wallis test); $^{\blacklozenge}$ represents a significant response ($P<0.05$, Wilcoxon's signed rank test).

caused the activation of other systems, which were responsible for the subsequent haemodynamic changes (Billiau & Vandekerckhove, 1991). However, if this were the case, one would expect TNF antibody pretreatment to abolish the haemodynamic changes observed during LPS infusion, but this did not happen.

It is notable that there were no consistent differences between the actions of the TNF antibodies with the g1 and g2a substituted Fc regions, despite the g1 TNF antibody being substantially better at preventing LPS-induced mortality in mice (Suitters *et al.*, 1994). Other groups have examined the effectiveness of TNF antibodies in several different models of endotoxaemia, and most reports agree that TNF antibodies are capable of reducing mortality rates in endotoxaemia (Beutler *et al.*, 1985a; Tracey *et al.*, 1987a; Mathison *et al.*, 1988; Hinshaw *et al.*, 1990; Suitters *et al.*, 1994). However, the ability of TNF antibodies to prevent the cardiovascular sequelae associated with endotoxaemia is not quite so clear. There are several reports indicating that TNF antibody treatment can protect against the development of rapid and substantial reductions in mean arterial pressure following administration of relatively large bolus doses of LPS (Mathison *et al.*, 1988; Thiemermann *et al.*, 1993), or bacteria (Tracey *et al.*, 1987a; Hinshaw *et al.*, 1990; Jesmok *et al.*, 1992). However, in a study of prolonged endotoxaemia in the baboon (Emerson *et al.*, 1992), TNF antibody pretreatment attenuated the early (2–4 h), transient reductions in mean arterial pressure and total peripheral resistance, but had no significant effect on haemodynamics over the subsequent 5 days. Schlag *et al.*, (1994) demonstrated that TNF antibody treatment, 4 h after the start of bacterial infusion in baboons, had no effect on haemodynamics during the first 24 h, and Fielder *et al.*, (1992) demonstrated that TNF antibody treatment, 30 min after LPS administration in rhesus monkeys, had no major effects on the haemodynamic profile observed. Interestingly, in the studies of Emerson *et al.*, (1992), Fielder *et al.*, (1992) and Schlag *et al.*, (1994), TNF antibodies conferred beneficial effects on mortality rates. Hence, it may be that the protective effects of TNF antibody treatment in endotoxaemia are independent of changes in haemodynamic status.

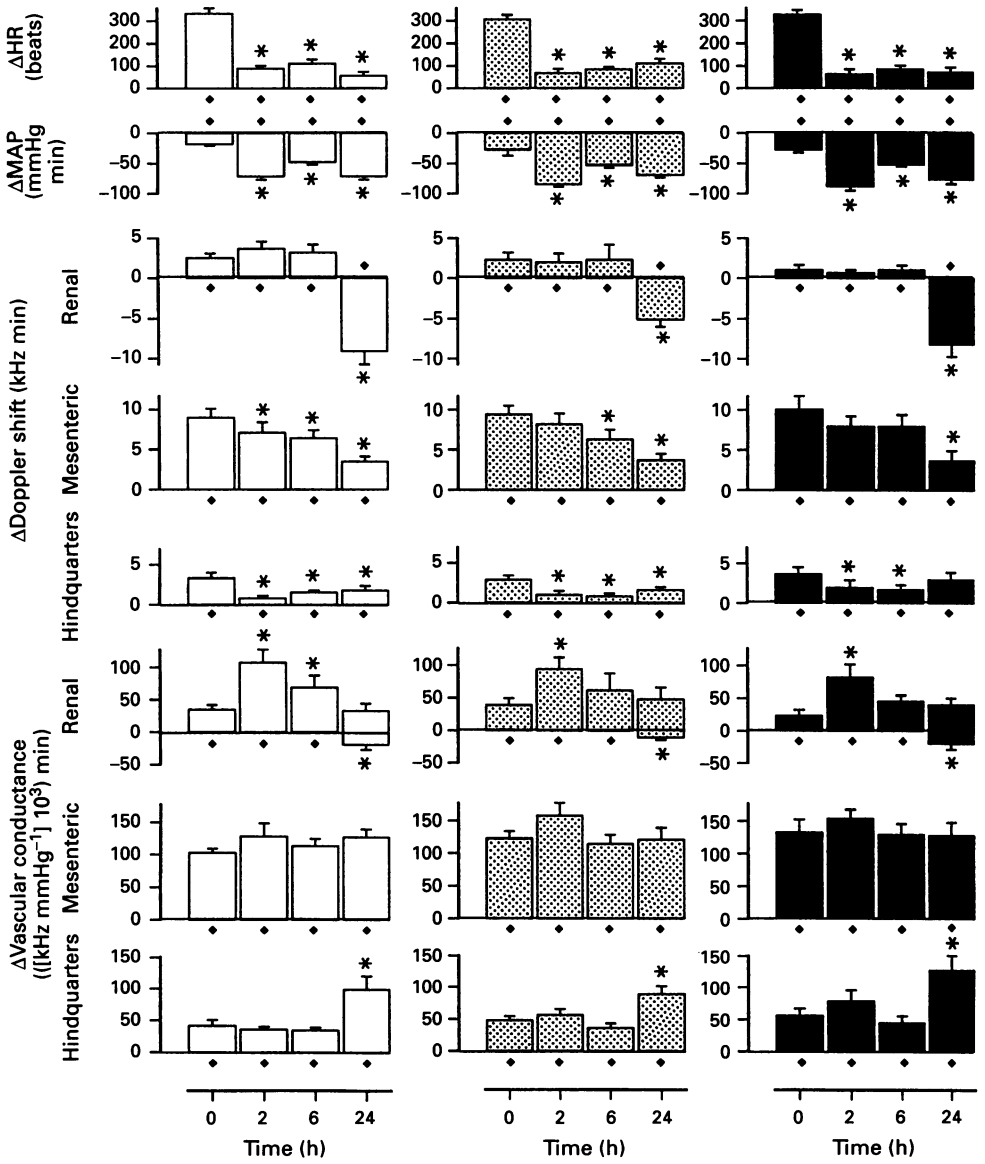

Figure 7 Changes (areas under or over curves 0–3 min) in heart rate (HR), mean arterial pressure (MAP) and renal, mesenteric and hindquarters Doppler shift and vascular conductance in response to bradykinin infusion (14.4 nmol min^{-1}) before and during a 24 h lipopolysaccharide infusion (150 μg kg^{-1} h^{-1}) after saline (open columns), TN3g1 (stippled columns), or TN3g2a (solid columns) pretreatment, in conscious Long Evans rats ($n=8$ in each group). Values are mean with s.e.mean, *$P<0.05$ versus time 0 (Friedman's test); ◆ represents a significant response ($P<0.05$, Wilcoxon's signed rank test).

Of the various vasodilator and vasoconstrictor challenges used, we found responses to acetylcholine were the most affected by antibody pretreatment, with the tachycardic response to acetylcholine being generally preserved, and its hypotensive effect after 24 h of LPS infusion not being enhanced. Previously, we (Waller *et al.*, 1994) suggested that the augmented hypotensive effect of acetylcholine after 24 h of LPS infusion may have been due to an inability of cardiac output to increase above pre-existing elevated levels (Gardiner *et al.*, 1994), since there was no enhanced hyperaemia in any of the vascular beds monitored. TNF is reported to have myocardial depressant actions *in vivo* (Natanson *et al.*, 1989; Eichenholz *et al.*, 1992) possibly through expression of the inducible isoform of NOS (iNOS) in the myocardium (Schulz *et al.*, 1992). Thus, suppression of the myocardial depressant actions of TNF, by the TNF antibodies, could explain why the hypotensive effect of acetylcholine, was normalized.

Overall, TNFAb administration conferred no protective effect on the development of hyporesponsiveness to methoxamine or salbutamol during LPS infusion. Previous studies have demonstrated that TNF antibodies can attenuate the development of hyporesponsiveness to noradrenaline in aortic rings taken from anaesthetized rats challenged with LPS (Thiemermann *et al.*, 1993). In that model, the hyporesponsiveness to noradrenaline is thought to be related to the induction of iNOS. However, we (Waller *et al.*, 1995) have reported that the development of vascular hyporesponsiveness to methoxamine in our model is unaffected by aminoguanidine, and hence may not involve iNOS.

It was suggested in our earlier study (Waller *et al.*, 1994) that the hyporesponsiveness to salbutamol may have been due to reduced β_2-adrenoceptor sensitivity (Auclair *et al.*, 1983; Van Heuven-Nolsen *et al.*, 1986), secondary to elevated circulating catecholamines (Chernow *et al.*, 1982; Schaller *et al.*, 1985). However, since it has been reported that TNF antibodies can attenuate the release of catecholamines after administration of bacteria (Tracey *et al.*, 1987a), our present findings indicate that the subsensitivity to salbutamol may be due to factors other than sympathoadrenal activation, or TNF release.

The haemodynamic responses to bradykinin, and the effect of LPS thereon, are difficult to interpret (Waller *et al.*, 1994), but they were essentially unaltered by pretreatment with both TNF antibodies. Hence, it seems that these changes are independent of the increase in plasma TNF. It is interesting that the tachycardic response to bradykinin, unlike that of acetylcholine, was not affected by the TNF antibodies.

The fact that endotoxaemia involves a complex interaction of numerous mediators which appear to have similar actions (see Billiau & Vandekerckhove, 1991, for review) is perhaps one explanation why removal of one mediator (i.e. TNF) in such a dynamic situation does not have dramatic effects on the haemodynamic profile observed. Overall, the results of the present study demonstrate that a continuous low dose infusion of LPS in the conscious rat results in a transient elevation of plasma TNF. Neutralization of plasma TNF with monoclonal antibodies has no consistent effect on resting regional haemodynamics and has only minor effects on the responses to a wide range of vasoactive agents.

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