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IL-8 as a circulating cytokine: induction by recombinant tumour necrosis factor-alpha

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SUMMARY

Tumour necrosis factor-alpha (TNF-α) is a pivotal cytokine at the centre of a cascade of cytokines and inflammatory mediators which modulate the host response to infection and trauma, and in particular the metabolic changes resulting in shock and subsequent multi-organ failure. The cytokine IL-8—predominantly an activator and chemotactic factor for circulating polymorphonuclear neutrophil leucocytes—is produced in response to TNF-α *in vitro*, and high circulating levels of IL-8 are found in septic primates. We have studied the release of IL-8 into the circulation of subjects with chronic hepatitis B undergoing a 10 week pilot trial of recombinant TNF-α (rTNF-α) therapy in doses of 15–100 $\mu g/m^2$. A marked dose-dependent increase in plasma IL-8 levels was seen commencing at 30–60 min after the start of rTNF-α infusion and peaking between 2 and 3 h (mean peak level 4300 ng/*l*). The temporal pattern of IL-8 production exactly echoed that of IL-6, another component of the cytokine cascade, but peak plasma levels of IL-8 were up to 17 times higher than those of IL-6. This study confirms *in vitro* data suggesting that IL-8 is a component of the acute circulating cytokine cascade with a potential role in the modulation of the acute immune and metabolic response to infection and trauma.

Keywords tumour necrosis factor IL-8 hepatitis B septic shock

INTRODUCTION

The production of tumour necrosis factor-alpha (TNF-α) in response to endotoxin has been shown to be of central importance in modulating many of the damaging metabolic changes associated with sepsis. High circulating levels of TNF-α correlate with mortality and tissue damage in sepsis and in other acute conditions associated with widespread multi-organ damage including severe burns [1], cerebral malaria [2] and fulminant hepatic failure (FHF)[3]. The central role of TNF-α is illustrated by experiments in which animals are challenged with normally lethal doses of endotoxin or live bacteria; MoAbs to TNF-α are protective in terms of mortality [4] and prevent the release of other cytokines [5] which modulate tissue damage, including IL-6 [6] and IL-1 [7].

The cytokine IL-8 is produced *in vitro* in response to TNF-α and is a potential mediator of the inflammatory response to infection and injury. IL-8 attracts and activates polymorphonuclear leucocytes *in vitro* [8] and *in vivo* [9] and inhibits leucocyte adhesion to activated endothelium [10]. IL-8 is generated by a variety of immune and non-immune cells, including macrophages and monocytes [8], endothelial cells [11], fibroblasts [12], hepatocytes [13] and polymorphonuclear leucocytes [14]. In addition to TNF-α a number of other cytokines result in IL-8 production *in vitro*, including IL-1, IL-2, IL-3 and GM-CSF [15]. Two studies now suggest that IL-8 may be released into the circulation as part of the acute inflammatory cytokine cascade. IL-8 is detectable in plasma following incubation of whole blood with endotoxin *in vitro* [16] and appears transiently in the circulation of primates with experimentally induced septic shock or following IL-1α infusion [17]. In the present study we show that the infusion of recombinant TNF-α (rTNF-α) in humans is associated with a dose-dependent increase in circulating levels of IL-8. The temporal relationship of IL-8 appearance to that of IL-6, another component of the acute inflammatory cascade [18], is also examined.

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PATIENTS AND METHODS

Four patients with chronic hepatitis B virus infection were treated with human rTNF-α (Eurocetus BV, Amsterdam, The Netherlands) in a phase one dose finding study to assess possible antiviral effects and toxicity [9]. All patients were positive for hepatitis B eAg and had evidence of chronic active hepatitis on a recent liver biopsy; one patient was cirrhotic. The patients were treated over 10 weeks with an escalating dose schedule of intravenous rTNF-α given daily for 5 days and then three times

weekly (Monday, Wednesday, Friday) for up to 10 weeks. TNF-α was administered intravenously as an infusion in 4% human albumin solution over 10–30 min. The dose of TNF-α was escalated every 2 weeks from a starting level of 10–15 μg/m² up to 100 μg/m² (via 30, 60 and 80 μg/m²). No serious toxicity was encountered with the exception of malaise and rigors which initially stopped after the first 3–4 infusions. All patients completed the full course of rTNF-α infusions up to the maximum dose of 100 μg/m² (two additional subjects were entered into the trial of rTNF but were unable to tolerate the treatment owing to persistent rigors). Malaise and rigors also recurred in two patients on the highest dose of rTNF-α (100 μg/m²), but were controlled with indomethacin pre-treatment. Ethical committee approval for the study was obtained.

Twelve studies were performed on the four patients at various stages during the course of therapy. These studies were performed with the first dose of rTNF-α (15 μg/m²), repeated after 1 week in one patient (15 μg/m²), and twice later in the course of treatment in all four patients (one study at dose 80 μg/m², seven studies at dose 100 μg/m²). After the initial studies, repeat studies were performed midweek, thus 48 h after the previous rTNF-α infusion. In three of the latter studies patients took indomethacin 25 mg 1 h before TNF-α infusion in order to reduce immediate side effects. Blood samples were obtained immediately before therapy and at varying intervals afterwards, in all studies blood samples were obtained at 1 h ($n=12$). Plasma cytokine levels were also assayed in 20 normal subjects to provide control data.

Cytokine assays

Peripheral blood was taken on ice into endotoxin-free heparinized glass tubes. The blood was spun at 600 *g* for 5 min and plasma separated and stored at −20°C until assayed. IL-8 were assayed using a sandwich ELISA (R&D Systems, Minneapolis, MN). Briefly, ELISA plates pre-coated with mouse monoclonal anti-human IL-8 were incubated for 2 h at room temperature with diluted plasma samples and a dilution range of recombinant human IL-8. The plates were washed three times (using PBS/0·05% Tween 20) and polyclonal antibody to human rIL-8 conjugated to horseradish peroxidase added for a further 2 h. The plates were washed again and, after 20 min incubation with stabilized tetramethylbenzidene, stopped with 2 N sulphuric acid and read at 450 nm. The ELISA detected IL-8 with a sensitivity of 18 ng/*l*, and intra and interassay variabilities of 9·2% and 12·2% respectively. There was no cross-reactivity in this ELISA with other cytokines and growth factors including: IL-1α or β, IL-2, IL-3, IL-4, IL-6, TNF-α or β, G-CSF, GM-CSF, TGF-β, PDGF or FGF. IL-6 and TNF-α were assayed as described previously using a sandwich ELISA [20]. For the purpose of standardization cytokine levels are quantified in mass terms; these do not necessarily reflect specific activity which is dependent upon the bioassay system used. Statistical analysis was by Mann–Whitney non-parametric testing (SPSS/PC+, Chicago, IL).

RESULTS

Plasma IL-8 was detectable at low levels in HBsAg-positive subjects before rTNF-α therapy (mean 63 ng/*l*, range 33-90), but was undetectable in normal control subjects. Following the initial rTNF-α infusion (15 μg/m²) a modest rise in plasma IL-8 levels was seen, commencing between 30 and 60 min after the start of infusion (mean 445 ng/*l*, range 278–646 at 60 min). In the subject where the study was repeated after 1 week of intermittent rTNF-α therapy using the same dose as given initially, the plasma IL-8 level before infusion was elevated (190 ng/*l*) but the level at 60 min was the same as in the initial study (412 ng/*l*). After several weeks of thrice weekly rTNF-α infusions in escalating doses, plasma IL-8 levels were increased before infusion in all subjects (mean 254 ng/*l*, 95% confidence interval (CI) 190–318, $n=8$). In each instance following rTNF-α (80–100 μg/m²) a marked rise in plasma IL-8 was seen commencing at 30–60 min peaking after 120–180 min and then falling with a circulating half life of around 60–90 min (Fig. 1). The mean plasma IL-8 level at 60 min was significantly higher (mean 1790 ng/*l*, 95% CI 1390–2190, $n=8$) than with the lower doses of rTNF-α. Pre-treatment with indomethacin had no effect on plasma IL-8 levels, levels at 60 min were essentially the same in untreated (mean 1800 ng/*l*) and indomethacin-treated subjects (mean 2000 ng/*l*).

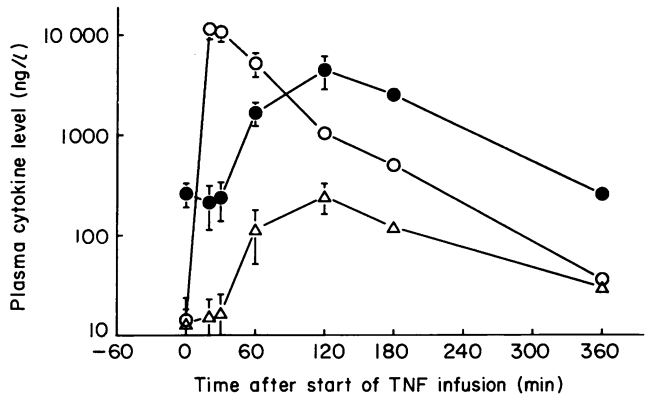

Fig. 1. Geometric mean plasma recombinant tumour necrosis factor-alpha (rTNF-α), IL-8 and IL-6 levels in ng/*l* after a dose of rTNF-α of 100 μg/m². Error bars (95% confidence limits) are given for each time point at which sufficient data were obtained. ○, TNF; ●, IL-8; △,IL-6.

IL-6 levels in these patients were previously reported [20]—essentially, the time course of cytokine production was identical for both IL-8 and IL-6 with both cytokines appearing at 30–60 min and peaking between 2 and 3 h after infusion. There were marked differences in the circulating levels of cytokines; peak levels of IL-8 (mean peak level 4300 ng/*l*, range 2200–6100) were approximately 17 times higher than those of IL-6 (mean peak level 295 ng, range 266–297) and around three times lower than those of rTNF-α following infusion (mean peak level 11 750 ng/*l*, range 5600–18 600, dose 100 μg/m², Fig. 1). No change was seen in peripheral neutrophil count over the course of therapy. However, full blood counts were obtained only every 24 h. Transient hourly changes in neutrophil counts would not therefore have been detected.

DISCUSSION

The present study demonstrates IL-8 is released into the circulation following rTNF-α infusion and confirms in humans the results of *in vitro* studies in which TNF-α has been found to be a potent inducer of IL-8 production [21]. Levels of IL-8 remained elevated during rTNF-α therapy despite the interval of 48 h between rTNF-α infusions. This may suggest that, after

initial stimulation with rTNF-α, other mechanisms prolong increased production of IL-8. This has also been reported with whole blood cultures *in vitro*, in which early (4–12 h) and delayed (24 h) phases of IL-8 production were identified following endotoxin stimulation [16]. The time course of IL-8 production *in vivo* and *in vitro* differs considerably, thus emphasizing the difficulty in extrapolating the results of even simple *in vitro* experiments to the more complex system present *in vivo*. With increased doses of rTNF-α a marked increase in IL-8 production was seen, strongly suggesting a dose-dependent relationship. As in this study subjects received the higher dose of rTNF-α only after several weeks of rTNF-α therapy, it is possible that with continued intermittent exposure to rTNF-α the IL-8 response is upregulated. However, this was not seen in the subject in whom the study was repeated with the initial dose of rTNF-α after the first week of treatment.

The plasma levels of IL-8 after rTNF-α infusion were very high and compare with the peak levels seen in septic primates [17] (up to 7000 ng/*l*) and in human subjects with FHF-induced multiple organ failure (up to 10 000 ng/*l*, unpublished data). In comparison the peak plasma levels of IL-6 after rTNF-α infusion were very much lower than the maximum levels of IL-6 (over 4000 ng/*l*) reported in sepsis [18] or in FHF-induced multiple organ failure [3]. Thus despite the very close temporal relationship between IL-8 and IL-6 production, it is clear that rTNF-α infusion does not reproduce the pattern of elevated circulating cytokine levels seen in critically ill patients.

It is striking that despite the pivotal role postulated for TNF-α in the clinical development of septic shock and other manifestations of endotoxaemia, rTNF-α infusion is not accompanied by severe toxicity in this and in other human clinical trials [22] despite the very high plasma TNF-α levels achieved. Similarly in animal studies, although the metabolic features of endotoxic shock are prevented by neutralizing anti-TNF-α antibodies, only when given in massive doses (e.g. 100 μg/kg for sheep [23]) will rTNF-α infusion alone cause severe tissue damage. Clearly the rTNF-α is biologically active within the circulation, as demonstrated in the present study by the profound induction of other cytokines, emphasizing the role of permissive or synergistic factors in modulating cytokine-mediated tissue damage. In this connection a number of factors including IL-1 [7], interferon-gamma [24], platelet activating factor [25] and IL-6 [6] have been shown to prime for or synergize with TNF-α in modulating tissue damage *in vivo*. Further studies are needed before it can be determined whether IL-8 is also implicated.

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