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Dose-dependent increase in plasma interleukin-6 after recombinant tumour necrosis factor infusion in humans

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SUMMARY

Several studies have shown that the cytokine interleukin-6 (IL-6) is produced in response to tumour necrosis factor (TNF) *in vitro*. This study examines the *in vivo* relation between these two cytokines with assays of plasma IL-6 and TNF levels in subjects with chronic hepatitis B undergoing immunomodulatory therapy with recombinant TNF (rTNF). Plasma IL-6 was detected from 20 min after rTNF infusion with levels peaking after 2–3 h and levels correlated with the dose of rTNF administered ($r = 0{\cdot}67$, $P = 0{\cdot}004$). Peak levels of IL-6 (mean 295, range 266–297 ng/*l*) were lower than those seen in certain disease states despite the very high peak levels of rTNF (mean 11 750, range 5623–18 620 ng/*l*). These findings suggest that the very high levels of IL-6 found in certain disease states are not purely the result of circulating TNF. Other factors such as endotoxin or other cytokines may also play a role in determining levels of plasma IL-6.

Keywords tumour necrosis factor interleukin-6 chronic hepatitis B

INTRODUCTION

The cytokine interleukin-6 (IL-6) is produced in a variety of tissues and has a multitude of immunoregulatory actions (Wong & Clark, 1988). IL-6 mRNA is induced in response to recombinant tumour necrosis factor (rTNF) *in vitro* (Zhang *et al.*, 1988) and high levels of circulating IL-6 have been found in several diseases, including sepsis (Waage *et al.*, 1989), burns (Childs *et al.*, 1989) and fulminant liver failure (N. Sheron, unpublished data). In each case the levels of IL-6 have correlated with those of rTNF, implying that either both are released as a result of a common stimulus or that circulating IL-6 is produced in response to circulating TNF. The latter is suggested by animal studies (Fong *et al.*, 1989) in which the administration of anti-TNF was found to prevent the subsequent appearance of circulating IL-6 and interleukin-1 (IL-1). In order to study the relation between circulating IL-6 and TNF *in vivo* we have assayed plasma IL-6 levels in patients with chronic hepatitis B being given rTNF therapy.

SUBJECTS AND METHODS

Five patients with chronic hepatitis B virus infection were treated with human rTNF in a phase-one dose-finding study to assess possible anti-viral effects and toxicity (Sheron *et al.*, 1990). The patients were treated over 10 weeks with an escalating dose schedule of i.v. rTNF given daily for 5 days and then three times weekly for up to 10 weeks. The dose was escalated every 2 weeks from a starting level of 10–15 $\mu g/m^2$ up to 100 $\mu g/m^2$, providing that no serious side effects were encountered with the previous dose level.

Fourteen studies were performed on four patients at various stages in their course of rTNF therapy. Four studies were performed after the first dose of rTNF (one patient 10 $\mu g/m^2$, three patients 15 $\mu g/m^2$), repeated after 1 week in two patients (15 $\mu g/m^2$), and later in the course of treatment (one at dose 80 $\mu g/m^2$, seven at dose 100 $\mu g/m^2$). In three of the latter studies the patients took 25 mg indomethacin prior to TNF infusion in order to reduce immediate side effects. For the purposes of this study rTNF was administered intravenously as an infusion in 4% human albumin solution. The infusion was given initially over a period of 20–30 min for the first and second doses but as the study progressed this was reduced, so that the higher doses of rTNF were given over a 10–15-min period. Blood samples were obtained immediately prior to therapy and at varying intervals afterwards, in all studies blood samples were obtained at 1 h ($n = 13$). Peripheral blood was taken on ice into endotoxin-free heparinized tubes (vacutainer). The blood was spun at 600 *g* for 5 min and plasma separated and stored at −20°C until assayed.

IL-6 was assayed with an ELISA (R&D Systems) with a sensitivity of 10 ng/*l*. TNF assays were performed using a radioimmunoassay (Medgenix) with a sensitivity of 5 ng/*l*. Data were analysed with an SPSS statistics program on a Dell 200 computer.

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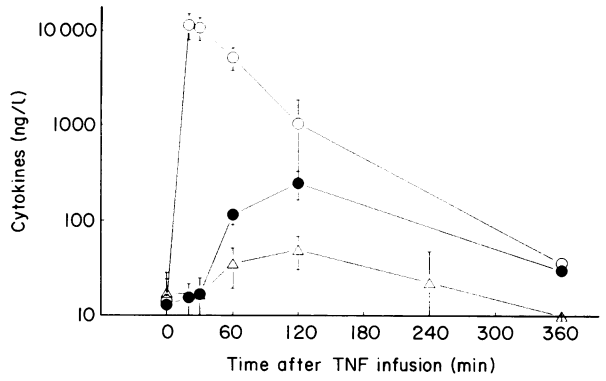

Fig. 1. Mean plasma tumour necrosis factor (TNF) and interleukin-6 (IL-6) levels in ng/l after a dose of recombinant TNF (rTNF) of 100 $\mu g/m^2$ (TNF, ○; IL-6, ●; $n=7$) and IL-6 levels (△, $n=6$) after a dose of 10–15 $\mu g/m^2$. Error bars (95% confidence limits) are given for each time-point at which sufficient data were obtained.

RESULTS

Plasma TNF concentrations peaked immediately following cessation of the infusion and declined thereafter (Fig. 1). With doses of rTNF of 100 $\mu g/m^2$, the peak level of plasma TNF was 11 500 ± 3000 ng/*l* (mean and 95% confidence interval) declining with a half-life of 40–50 min. Plasma IL-6 was detected at low levels (range 10–45 ng/*l*) in plasma prior to rTNF infusion on six out of 14 occasions. A rise in the level of plasma IL-6 was seen between 30 and 60 min after the start of TNF infusion (Fig. 1), rising to a maximum after 2 h and then declining with a half-life of 30–70 min.

Plasma levels of both TNF and IL-6 at 1 h were dependent on the doses of rTNF infused (for TNF $r=0{\cdot}76$, $P=0{\cdot}002$; and for IL-6 $r=0{\cdot}67$, $P=0{\cdot}004$) and there was a significant correlation between TNF and IL-6 levels at this time ($r=0{\cdot}55$, $P=0{\cdot}03$). Peak levels of TNF (mean 11 750 ng/*l* at 100 $\mu g/m^2$, range 5623–18 620) were considerably higher than those of IL-6 (mean 295 ng/*l* at 100 $\mu g/m^2$, range 266–297).

There was no change in the mean 1-h plasma IL-6 level in the two patients in whom the study was repeated with the same dose of rTNF after 1 week of therapy (32·5 and 35 ng/*l*, respectively). In the three studies in which the patient had pre-treatment with indomethacin, the levels of plasma IL-6 tended to be slightly lower for an equivalent dose of rTNF (mean 97 *versus* 137 ng/*l*), although the numbers in each case are too small for this data to be of significance.

DISCUSSION

The present studies show clearly that IL-6 is released into the circulation in response to rTNF administration. IL-6 was detectable in plasma 20 min after the start of TNF infusion and reached a maximum after 2–3 h. The level of plasma IL-6 was related to the dose of rTNF administered, although in this study patients received the higher dose only after several weeks of rTNF therapy. It is possible therefore that with continued exposure to rTNF the IL-6 response is up-regulated. This seems less likely than a dose-related response and was not seen in the two patients in whom the study was repeated with the same dose of TNF after a week of therapy. Our findings support *in vitro* data (Zhang *et al.*, 1988), and confirm the results of the previous study in which circulating IL-6 was found in response to rTNF *in vivo* (Jablons *et al.*, 1989). In the latter study, elevated levels of plasma IL-6 were found following both rTNF and rIL-2 infusion although no dose–response studies were performed.

The peak levels of IL-6 seen in this study are considerably lower than the peak levels of IL-6 found in a number of disease states (Waage *et al.*, 1989). In a recent study of patients with fulminant hepatic failure (N. Sheron, unpublished data), levels of both TNF and IL-6 were elevated with a significant correlation between the two, but the peak levels of IL-6 (> 4000 ng/*l*) were much higher than the peak levels of TNF (< 150 ng/*l*), whereas in the present study peak levels of IL-6 (297 ng/*l*) were markedly lower than the peak levels of TNF (18 000 ng/*l*). The data would suggest, therefore, that although there is a dose-dependent increase in plasma IL-6 in response to circulating TNF, in disease states other factors such as cytokines or bacterial toxins may also be important in modulating IL-6 production.

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