

CIRCULATING INTERLEUKIN 6 DURING A
CONTINUOUS INFUSION OF TUMOR NECROSIS
FACTOR AND INTERFERON γ

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IL-6 (reviewed in references 1-3) is a recently recognized cytokine that is identical to the 26-kD protein, IFN- β_2 , B cell-stimulating factor 2, hybridoma growth factor, and hepatocyte-stimulating factor. Its pleiotropic functions include actions on lymphocytes, hepatocytes, and hematopoietic progenitors. It has been shown in vitro and/or in vivo in mice that LPS, TNF, IL-1, and platelet-derived growth factor can induce the synthesis and/or release of IL-6.

It is conceivable that IL-6 is the mediator of some of the actions that are observed after the in vivo administration of TNF or IL-1, such as the induction of at least part of the acute phase response, and it has been suggested that IL-6 is implicated in other human diseases such as autoimmune disease.

Consequently, it would be useful to know whether TNF also induces IL-6 in human patients and, if so, to what extent. Moreover, it would be of interest to know whether such in vivo synthesis is modulated by other substances, such as IFN- γ , which are synergistic with TNF. To that end, we analyzed plasma samples obtained during phase I clinical trials involving a 24-h continuous infusion of human recombinant (hr)TNF (4) and of two overlapping, 24-h continuous infusions of hrIFN- γ and hrTNF (5).

Materials and Methods

Patients. Plasma samples of 27 cancer patients were included. 16 patients had been admitted to the Dana-Farber Cancer Institute (Boston, MA) for treatment during a phase I clinical trial of hrTNF administered as a 24-h continuous intravenous infusion. Details of inclusion and exclusion criteria, tumor responses, experimental protocol, toxicity patterns, and metabolic responses have been reported elsewhere (4, 6). Written consent was obtained from each patient and the studies were approved by the hospital's Institutional Review Board.

The 11 other patients were admitted to the same institute for treatment during another phase I clinical trial (5) of two overlapping, 24-h continuous infusions comprising a fixed dose of hrIFN- γ (200 $\mu\text{g}/\text{m}^2$) and a variable dose of hrTNF (ranging from 4.5 to 205 $\mu\text{g}/\text{m}^2$); the latter infusion started 12 h after the onset of the first. Exclusion and inclusion criteria, as well as treatment conditions, were essentially the same as in the first trial (5). hrTNF was

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obtained from Asahi Chemical Industry, New York, NY, and hrIFN- γ from Amgen, Thousand Oaks, CA. The hrTNF infused contained <10 pg endotoxin/mg protein as assessed by the limulus assay.

Samples. Blood was sampled with a forearm venous catheter or through direct venipuncture, immediately before the infusion and after 0.5, 1, 3, 6, 12, and 24 h. The samples were stored as plasma at -80°C . In the case of the combined infusion, blood was sampled immediately before the infusion and after 6, 12, 13, 15, 18, 24, and 36 h (i.e., 12, 6, and 0 h before the onset of the hrTNF infusion, and 1, 3, 6, 12, and 24 h during the infusion).

Bioassay. IL-6-dependent mouse hybridoma cells, namely 7TD1 cells (7), were cultured in flat-bottomed 96-well microtiter plates (7,000 cells/well) in the presence of medium, serial dilutions of plasma, or purified, yeast-expressed hrIL-6 (250 pg/ml) as a standard. After 3 d of culture, the number of surviving cells was determined by a colorimetric assay for hexosaminidase (8). Titers were assigned by comparing dilutions of samples and of the standard needed to obtain half-maximal growth of the hybridoma cells; they are expressed as the logarithm of the concentration in picograms/milliliter. The detection level (half-maximal growth of 7TD1 cells) for hrIL-6 was 2–4 pg/ml. The detection level of IL-6 in human plasma was, due to the toxicity of some undiluted plasma samples for the tester cells, arbitrarily set at 40 pg/ml. In some experiments a polyclonal rabbit antiserum raised with yeast-expressed hrIL-6, purified to near homogeneity (2), was added to test the specificity of the growth factor activity. The presence of TNF, IFN- γ , or IL-1 did not interfere in the assay.

Results

We analyzed plasma from 16 patients receiving a continuous, 24-h infusion of hrTNF, sampled before the infusion and at various time points during the infusion. None of the plasma samples taken before the infusion contained detectable amounts of IL-6 (<40 pg/ml). All patients (except for the two receiving the lowest doses of hrTNF, namely 22 and 45 $\mu\text{g}/\text{m}^2$, where TNF levels were not detected) transiently had detectable IL-6 levels, the major peak levels being found in plasma collected after 6 h ($n = 12$) (Fig. 1). Only two patients showed a peak IL-6 level already 3 h after the onset of the infusion. The time of the peak IL-6 level for each patient can be seen in Fig. 2. Fig. 1 compares the kinetics of the presence of TNF and IL-6 detectable in the plasma (results of TNF determinations in patients of this phase I trial have been published previously [4, 6] and are displayed here only to show the time relationship with the presence of IL-6). Because of the considerable interindividual variation in detectable hrTNF concentrations, levels are shown for a hypothetical average patient calculated as the means from patients over the whole dose range of hrTNF administered. It can be observed that even during a continuous infusion of TNF, both cytokines are only transiently detectable in the plasma and that the wave of detectable TNF precedes the wave of detectable IL-6, which persists for a somewhat longer time.

The samples containing the peak IL-6 levels were retested in triplicate in one assay run to exclude interassay variability (Fig. 2). For hrTNF doses between 136 and 545 $\mu\text{g}/\text{m}^2$, a good correlation exists between the dose of hrTNF and the level of IL-6 obtained ($y = 2.14 + 0.004 x$; $r = 0.86$; $p < 0.001$). To know if an IL-6 plateau value is reached with high doses of hrTNF, more patients should be included in the test. This is, due to the toxicity of the treatment, not realistic. Addition of antiserum to hrIL-6 completely neutralized the activity in the plasma samples, documenting the specificity of the bioassay.

We also analyzed plasma samples from 11 patients receiving two overlapping 24-h infusions of hrIFN- γ (fixed dose of 200 $\mu\text{g}/\text{m}^2$) and hrTNF, the latter starting 12 h

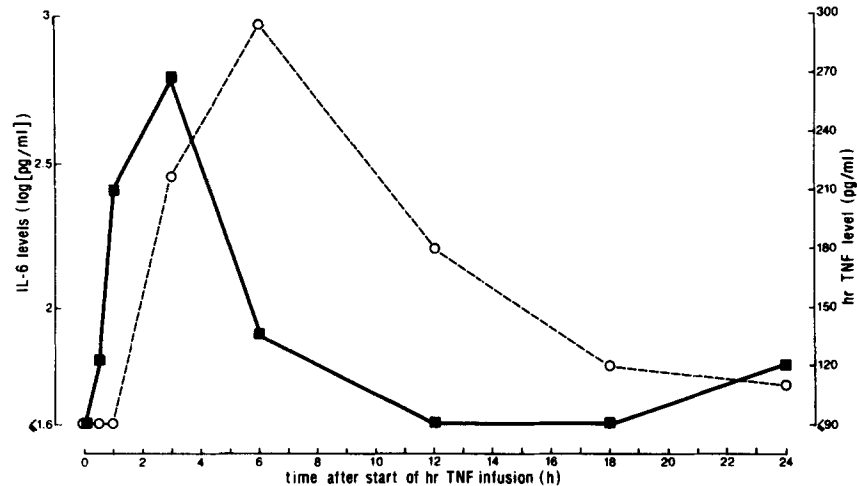


FIGURE 1. Comparison of kinetic profiles of detectable IL-6 and TNF during a 24-h continuous infusion of hrTNF. Values of detectable IL-6 and TNF levels are plotted against time for a hypothetical average patient having levels calculated as the means from patients over the whole dose range of hrTNF administered. Mean IL-6 levels (O) are calculated based on data from 16 patients. Mean TNF levels (■) are based on data from 12 patients receiving hrTNF over 24 h, and were determined by ELISA. Details and data of these TNF determinations have been published previously (4). To calculate the mean values, results below the detection level were assigned a theoretical value of half of the detection level (detection levels are 40 pg/ml for IL-6 [$\log 40 = 1.6$] and 90 pg/ml for TNF). Note that the IL-6 levels (*left ordinate*) are expressed as the logarithms of the concentration.

after the onset of the infusion with hrIFN- γ . Before the onset of the infusions with hrTNF, no IL-6 could be detected, except for two samples containing low values of IL-6: a plasma sample obtained after 6 h contained 113 pg/ml of IL-6, while a sample from another patient taken after 12 h contained 59 pg/ml of IL-6. As for the trial without hrIFN- γ , a transient appearance of IL-6 was detected for all patients after the onset of the infusion with hrTNF, except for the one who received the lowest dose of TNF, namely 4.5 $\mu\text{g}/\text{m}^2$. The samples per patient containing the peak IL-6 levels were reassayed in the single assay run mentioned above (Fig. 2). Linear regression analysis also showed a good correlation between the IL-6 values obtained and the hrTNF dose received in the combination study ($y = 1.644 + 0.012x$; $r = 0.96$; $p < 0.001$).

When the results obtained for the second part of this study are compared with those of the first part, two differences can be observed. First, the peak levels of IL-6 recorded for a same dose of hrTNF are higher in the case of the treatment involving IFN- γ also. Second, the time of the peak is more variable than in the first series: 1 h ($n = 2$), 3 h ($n = 3$), and 6 h ($n = 5$) after the onset of the infusion with hrTNF, the correlation being nil between the time that the peak level is reached and the dose of hrTNF is given. It should be noted that the linear regression calculation based on the first study involving patients receiving hrTNF alone and considering only a comparable dose range of hrTNF (namely 136–227 $\mu\text{g}/\text{m}^2$), yields a regression line whose slope is very similar to the one obtained for the results from patients receiving the combination of hrTNF and hrIFN- γ ($y = 0.738 + 0.012x$; $r = 0.91$; $p < 0.05$).

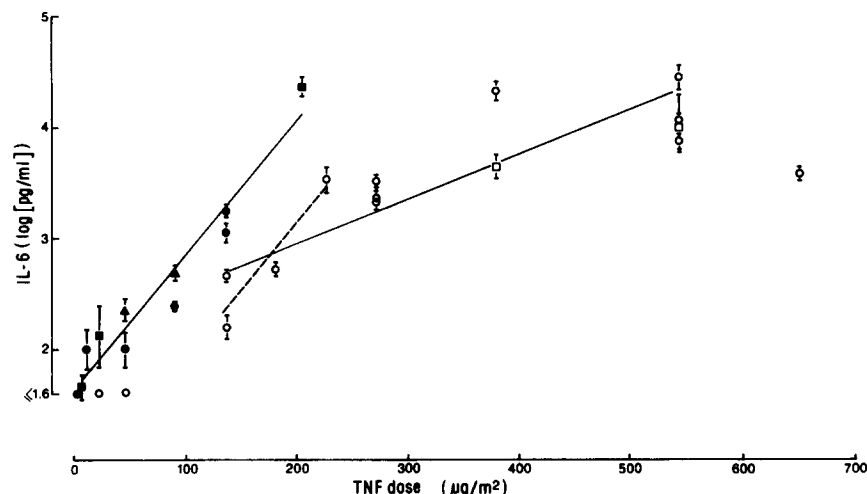


FIGURE 2. Correlation between hrTNF dose and peak IL-6 levels. Logarithmic means \pm SD of triplicate determinations of peak IL-6 levels for each patient are plotted against the hrTNF dose administered. Open symbols refer to patients who were treated with hrTNF alone (\circ , peak level reached at 6 h; \square , peak level reached at 3 h after the start of the infusion). A linear regression was calculated by the least squares method for the dose range between 136 and 545 $\mu\text{g}/\text{m}^2$ ($y = 2.14 + 0.004 x$; $r = 0.86$; $p < 0.001$). Closed symbols refer to patients who were treated with overlapping infusions of hrIFN- γ (fixed dose of 200 $\mu\text{g}/\text{m}^2$) and hrTNF (\blacktriangle , peak level reached after 1 h; \blacksquare , peak level reached after 3 h; \bullet , peak level reached after 6 h; hours after the start of the infusion with hrTNF). Linear regression was calculated for the dose range between 5.45 and 205 $\mu\text{g}/\text{m}^2$ ($y = 1.644 + 0.012 x$; $r = 0.96$; $p < 0.001$). A third linear regression was calculated using the IL-6 levels of patients receiving hrTNF alone in the dose range between 136 and 227 $\mu\text{g}/\text{m}^2$, i.e., that part of the dose range that is comparable with the hrTNF dose range of patients receiving the combination of hrIFN- γ and hrTNF and yielding IL-6 levels above the detection limit ($y = 0.738 + 0.012 x$; $r = 0.91$; $p < 0.05$).

Discussion

The results show that TNF administered to patients can induce considerable amounts of IL-6 in the plasma. Consequently, TNF, in addition to its direct effects, may also indirectly induce an acute phase reaction, activate the immune system, and exert other pleiotropic activities.

The fact that the appearance of IL-6 in plasma is only transient during a continuous infusion may be ascribed to two reasons. First, it is possible that the *in vivo* induction of IL-6 by TNF belongs to the burst-like type or that negative feedback-regulating mechanisms are also induced, either by TNF or by IL-6. Alternatively, this transient effect could be due to the so far unexplained fact that TNF does not reach a steady state level during continuous infusion (4, 9), but can only be detected transiently in the circulation.

The observation of higher levels of IL-6 during the combined infusion test is not unexpected, since IFN- γ is synergistic with TNF for other effects, such as antitumor activity in mice (10) and other biological activities in this trial (5). The fact that the regression lines of both treatments had a comparable slope for the lower dose range of hrTNF could indicate that the mechanism of IL-6 induction is similar in both cases, and that the higher amounts of IL-6 found in the case of the combined

treatment could be ascribed to a sensitization of the target cells to hrTNF, e.g., by augmenting the number of receptors. The observation that this slope is steeper than the one of the overall regression line for the treatment with hrTNF alone makes it likely that a plateau value of IL-6 would be obtained for higher doses of hrTNF.

It has been shown that there is a good correlation between the dose of hrTNF administered in this trial and the level of C-reactive protein obtained (6). The present observation that there is also a good correlation between the hrTNF dose administered and the IL-6 level obtained is in accordance with the hypothesis that the acute phase reaction observed after administration of hrTNF to patients is mediated by IL-6.

It has been proposed that TNF is the agent responsible for endotoxic shock, and there is indeed extensive evidence that TNF is a necessary key factor in septic shock (11). It should be noted, however, that not only after LPS do a number of cytokines appear in blood, but that this is also the case after TNF. Hence, it is conceivable that the shock observed after LPS, which can be mimicked by TNF in animals, is the result of a complex interplay of different cytokines. The synergistic/antagonistic effects of these induced cytokines (like IL-1 and IL-6) in different clinical situations remain to be evaluated. Recent results in animal models indeed suggest a synergistic role for IL-1 in endotoxic shock (12), whereas our preliminary results in mice do not show an enhancement of toxicity by IL-6.

Summary

Plasma samples obtained from patients receiving a 24-h continuous infusion of human recombinant (hr)TNF or a combination of two overlapping, 24-h continuous infusions of hrIFN- γ and hrTNF were analyzed for IL-6 in a sensitive bioassay. A transient appearance of circulating IL-6 was observed with peak levels between 3 and 6 h after the start of the hrTNF infusion. These peak levels correlated quite well with the dose of hrTNF administered ($r = 0.86$; $p < 0.001$). The maximal value observed was 27.5 ng/ml IL-6 in a sample of a patient receiving 545 $\mu\text{g}/\text{m}^2$ hrTNF. The combination of hrIFN- γ (200 $\mu\text{g}/\text{m}^2$) and hrTNF in the infusions resulted in higher IL-6 levels than a comparable dose of hrTNF alone. A maximal value of 23.5 ng/ml IL-6 was observed in a patient receiving 205 $\mu\text{g}/\text{m}^2$ hrTNF. No IL-6 was found in the plasma of patients during the 12-h infusion with hrIFN- γ alone, except for two borderline samples.

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