

Identification of TNF-LT Blocking Factor(s) in the Serum and Ultrafiltrates of Human Cancer Patients

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ABSTRACT

Human serum ultrafiltrate (UFS) obtained from cancer patients contains material(s) which inhibit the cytolytic activity of tumor necrosis factor (TNF-alpha) and lymphotoxin (LT-TNF-B) in vitro. These factors are found in UFS from patients with different types of cancers and are not detected in the sera of normal donors. Results from molecular sizing studies demonstrated that their molecular weights are over 30 kD. They appear to block by interfering with the early stages of cell lysis. It is not clear whether or not these TNF-LT blocking factor(s) are the same molecules. These factors may have adverse effects on the biological activities of TNF and LT in cancer patients.

INTRODUCTION

Tumor necrosis factor (TNF) and lymphotoxin (LT) are two related cytokines which can be released by stimulated lymphocytes and macrophages. LT is primarily a product of lymphocytes [1], whereas TNF can be released by both lymphocytes and macrophages [2]. These proteins have now been shown to cause a wide range of effects on cells in vitro and on tissues in vivo [3].

It is natural to expect that such active molecules are controlled by various mechanisms in vivo. One possible mechanism was recently reported by Peetre, et al [4], who reported the presence of a factor(s) in the serum and urine of patients on hemodialysis treatment which bound to TNF in vitro.

A phase I clinical trial has been conducted in which the blood of cancer patients has been ultrafiltered to remove immune suppressive factors of less than 100 kD [5]. In the present study, we have found that the ultrafiltrate (UFS) contains inhibitors of the cytolytic activity of recombinant human TNF and LT in vitro. These materials were detected in the UFS of all cancer patients but not in materials from normal donors. These factors may block the activities of TNF and LT in the cancer patient.

MATERIALS AND METHODS

Preparation of UFS from Various Donors

The UFS was obtained from Dr. Rigdon Lantz at the John Kennedy Hospital, Indio, California. The ultrafiltration procedure has been described previously [5]. All patients employed in these studies had advanced stages of cancer and had failed all traditional therapies. They had not received chemotherapy for four months prior to ultrafiltration. These individuals are Na--breast adenocarcinoma, Ha, Ma, Js, Vc--adenocarcinoma of the prostate, Gm--glioblastoma multiforma, Es--melanoma and Av--larynx squamous cell carcinoma. Normal controls were derived from ultrafiltrate blood samples from normal donors.

TNF or LT Assay

Recombinant human TNF or LT (Genentech Corp., South San Francisco, CA) activity were assayed with L929 mouse fibroblast by method of Granger, *et al* [6].

UFS from the sera of various cancer patients or normal donors was added along with the TNF or LT in the above assay. Percent inhibition of TNF or LT activity was calculated by using the following formula:

% Inhibition =

$$\frac{\text{Killing \% of LT or TNF} - [\text{Killing \% (LT or TNF + UFS)}]}{\text{Killing \% of LT or TNF}} \times 100$$

Molecular Sizing Assay

UFS was dialyzed against 0.15 M NaCl, 0.01 M phosphate, pH 7.2, using various sizes of dialysis tubes (Spectra/POR, USA) at 4°C for 12 hrs. Various dilutions of these samples were then assayed for inhibition activities.

RESULTS

Samples (10-100 µl) of serum and ultrafiltrate from a total of ten normal and eight cancer patients were tested for their ability to inhibit 0.01 to 100 ng of TNF and LT activity *in vitro*. A summary of some of these results is given in Figure 1. UFS from cancer patients showed inhibition of both TNF and LT activity. 79% of TNF activity and 48% of LT activity were inhibited by the UFS from the cancer patients, while the UFS from normal donors did not show significant inhibition of either TNF or LT.

Two separate sets of studies were established: one in which LT and TNF levels were held constant and the volume of ultrafiltrate was varied; and another in which ultrafiltrate was held constant and levels of TNF and LT varied. A summary of the results of the latter studies is shown in Figure 2.

There is a concentration dependence of both LT and TNF on the inactivating activity in serum ultrafiltrate. While the data are not shown, similar results were observed when TNF and LT were held constant and the ultrafiltrate was varied.

The UFS was dialyzed using two molecular size cut dialysis tubes, 10 kD and 30 kD. The inside and outside solutions of these dialyzed tubes were tested for inhibitory activity (see Figure 3).

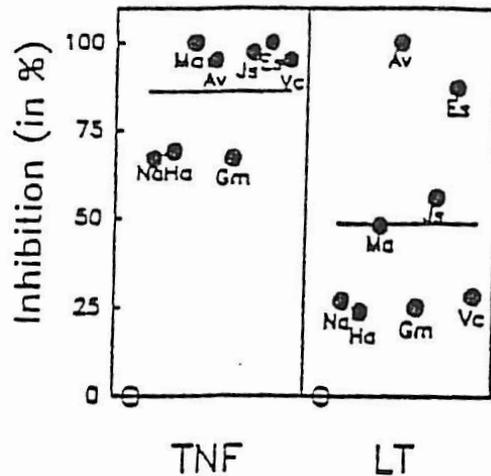


FIGURE 1: Identification of TNF-LT blocking factor(s) in the serum ultrafiltrate of human cancer patients. Ultrafiltrates were held constant (25 μ l). UFS was tested for its ability to inhibit TNF (0.8 ng) and LT (0.2 ng) activity.

- : Blocking activity of normal donor's group.
- : Blocking activity of each cancer patient's USF.
- : Average of cancer patient's blocking activity.

Inhibitory activity was recovered only in the inside solution. The molecular weight of these inhibitory factors therefore seems to be more than 10 kD.

DISCUSSION

The serum ultrafiltrates from cancer patients contained TNF

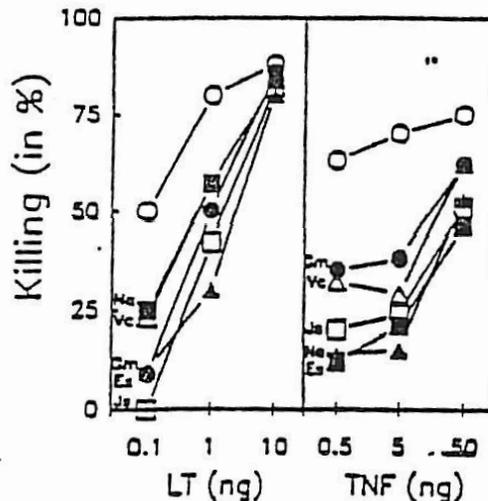


FIGURE 2: Stoichiometry of TNF-LT blocking activity. Ultrafiltrates were held constant (25 μ l) and levels of TNF and LT varied.

- : Normal donor's UFS
- The other symbols indicate the UFS of each cancer patient.

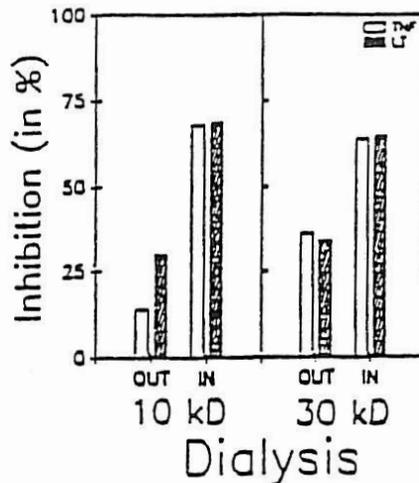


FIGURE 3: Molecular sizing assay.
The UFS (Aa) was dialyzed using two molecular size tubes (10KD,30KD).

and LT inhibitory factors. TNF inhibitory factors in the urine of certain febrile patients were previously reported [4, 7]. These authors suggested that these factors regulate TNF activity in these patients [4]. We found inhibitory factor(s) for both TNF and LT in the UFS of cancer patients. It is not clear whether these TNF and LT inhibitory factors are the same or distinct molecules.

Our studies indicated that the inhibitor(s) of LT and TNF are macromolecules. While the data are not shown, dialysis data has been extended to gel filtration and ultrafiltration. Collectively, these results indicate the inhibitory activities are between 10-50 kD, Peetre, *et al*, reported that the molecular weight of urine TNF binding factor is 30 kD [8]. The stoichiometry of blocking TNF and LT suggests direct relationship between the amount of blocker and the amount of TNF and LT. Purification and amino acid sequences are necessary to determine if these inhibitors are related.

TNF and LT are controlled by various mechanisms *in vivo*. For example, they are inducible, their production is inhibited by prostaglandin E2 [9] and the effect of TNF is inhibited by hydrocortisone [10]. TNF and LT inhibitory factors may be another mechanism for regulation.

TNF and LT have anti-tumor effects *in vitro* and *in vivo*, but they show only weak anti-tumor effects in clinical use. This situation may be explained by the existence of inhibitory factors which block their activity. The approach of ultrafiltrating therapy in cancer patient is to eliminate these factors.

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