Tumor Necrosis Factor in Children with Malignancies

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ABSTRACT

We measured serum tumor necrosis factor α (TNF) concentrations by a double-antibody radioimmunoassay method, with a detection level of 10 ng/liter, in 32 children with malignancies. Seventeen had acute lymphoblastic leukemia, 4 had acute nonlymphocytic leukemia, and 11 had solid tumors. At the diagnosis of malignant disease, 30 of the 32 patients had elevated serum TNF levels ranging up to 450 ng/liter. After complete remission status was achieved, 2–6 months from the diagnosis, the TNF levels were within the range of 130 healthy children who served as the reference group. Most of them had TNF levels below the detection limit. We consider the upper limit of normal to be 40 ng/liter. We conclude that elevated serum TNF concentration may be of potential significance in the diagnosis and follow-up of children with malignant diseases.

INTRODUCTION

TNF is a product of mononuclear macrophages (1, 2) and is produced in greatest quantity after exposure to bacterial endotoxins. The production may also be stimulated by other infectious agents, e.g., by viruses (3, 4) or protozoa (5–8). TNF is a mediator of general inflammation and is one of the endogenous pyrogens (9). Furthermore, TNF is a primary mediator in endotoxic shock, although the precise mechanism is unknown. The name “tumor necrosis factor” emerges from an early observation that certain severe bacterial infections may lead to hemorrhagic necrosis of a concurrent malignant tumor (10). TNF also has antitumor effect against several types of human tumor cells in vitro (11–13) and in human tumor xenograft models (14, 15). The hemorrhagic necrosis of tumors is suggested to originate in changes in vascular endothelium and in altered hemostatic properties caused by TNF (16, 17). These changes may also lead to systemic disseminated intravascular coagulation.

The hypothesis that TNF might be an endogenous antineoplastic agent is contradicted by studies regarding undetectable TNF levels in cancer patients (18–20). However, different results have been obtained by using more sensitive methods (21–23). Balkwill et al. (22) found TNF-like activity in the sera of patients with active cancer of several types of human tumor cells in vitro (11–13) and in human tumor xenograft models (14, 15). The hemorrhagic necrosis of tumors is suggested to originate in changes in vascular endothelium and in altered hemostatic properties caused by TNF (16, 17). These changes may also lead to systemic disseminated intravascular coagulation.

Several phase I clinical trials have been performed by utilizing recombinant TNF in patients with advanced cancer. These trials have revealed significant toxicity but poor objective anticancer responses (19, 24–30).

We wanted to measure serum TNF levels in children with a spectrum of malignancies, in order to see whether detectable TNF levels could be found by our current sensitive radioimmunoassay method, and to find out whether the TNF levels would reveal any diagnostic or prognostic significance.

SUBJECTS AND METHODS

Patients. Our series comprised 32 children with malignant disease, diagnosed consecutively at the Children’s Hospital, University of Helsinki, Finland. Twenty-one patients had acute leukemia, and 11 had pediatric solid tumors. The patients were of age 1–15 years. Seventeen were male and 15 were female.

Of the 17 children with ALL, 6 were of standard, 5 of intermediate, and 3 of high risk (31), two had lymphoma-leukemia, and one was Burkitt’s leukemia with L3 morphology. The induction therapy for ALL followed the Scandinavian ALL protocol (31), consisting of prednisone, vincristine, doxorubicin, L-asparaginase and intrathecal methotrexate in standard risk; additionally cyclophosphamide, cytotoxic arabinoside, and 6-mercaptopurine plus daunomycin instead of doxorubicin in intermediate risk; and in addition i.v. methotrexate and VM-26 in high risk. The lymphoma-leukemias were treated according to the LSA2-L2 protocol (32), and the Burkitt’s leukemia was induced on a modified Ziegler protocol (33).

Four children had ANLL, one with M2, one with M3, and two with M5 FAB types. They were induced on three- to five-drug conventional ANLL chemotherapy regimens.

The 11 patients with pediatric solid tumors received multi-agent combination chemotherapy, four for Wilm’s tumor (34), three for osteosarcoma (35), one for rhabdomyosarcoma (36), one for malignant histiocytosis (37), one for neuroblastoma, and one for Askin tumor (small round blue cell tumor of chest wall in childhood).

Reference Subjects. We had serum samples from 130 healthy subjects, 72 males and 58 females, age 1–15 years (median, 3 years), seen 1 month after recovery from acute lower respiratory tract infections (38). They had no underlying basic illness.

Laboratory Methods. Venous blood was drawn for this study at diagnosis and at 2, 4, 6, 8, 10, 12, 16, 20, and 24 weeks, in association with other routine blood sampling. The sera was kept frozen at −20°C until analyzed.

The concentrations of TNF in serum were measured by a double-antibody radioimmunoassay (39). TNF in serum competes with a fixed amount of 125I-labeled TNF for the binding sites of specific rabbit antibodies. The bound TNF is precipitated with Sepharose-bound antirabbit IgG and then centrifuged, and the radioactivity of the pellets is counted. As standard we used Escherichia coli-derived recombinant human tumor necrosis factor α, obtained from Dr. G. R. Adolf, Ernst-Boehringer-Institut fur Arzneimittelforschung (Wien, Austria). It had a specific activity of 6 × 10⁶ units/g of protein, as measured by bioassay with mouse L 929 cells. Rabbit antiserum to human TNF (Genzyme Corporation, Boston, MA) used in this study is directed to the intact recombinant M, 36,000 human tumor necrosis factor α. It showed no cross-reaction with lymphotoxins (tumor necrosis factor δ), interleukin 1, interleukin 2, or interferons. The detection limit of the assay is 10 ng/liter.

Accordingly, we were measuring tumor necrosis factor α molecule concentrations. Since we did not run parallel bioassays, we cannot guarantee that these TNF molecules always were functional.

Statistical Methods. The nonparametric Mann-Whitney’s U test was used in the statistical analyses.

RESULTS

Our data show that 30 of 32 children with newly diagnosed malignancies had elevated serum TNF values. In children with ALL and ANLL, the TNF levels were consistently high at diagnosis (Fig. 1). In children with solid tumors, greater variability was observed, with even undetectable TNF levels in an occasional patient (Fig. 1).
risk); 4. parameningeal rhabdomyosarcoma. The lower limit of detection is 10,
and the defined upper limit of normal is 40.

Fig. 1. Tumor necrosis factor α levels in serum of children with different newly diagnosed malignant diseases. Wilms, Wilms' tumor; Osteo, osteosarcoma. 1, Malignant histiocytosis; 2, Askin tumor; 3, neuroblastoma (stage III, poor risk); 4, parameningeal rhabdomyosarcoma. The lower limit of detection is 10,
and the defined upper limit of normal is 40.

The TNF levels at diagnosis did not correlate with the presence or absence of fever and infection. Eight children febrile over 38°C (axillary) had a median TNF of 133 ng/liter, compared to 24 nonfebrile children with a median TNF of 140 ng/liter (not significant).

The high TNF levels at the diagnosis of malignant disease subsequently decreased into the reference range (P < 0.01) after complete remission status was achieved (Fig. 2). The same phenomenon was observed both in leukemia and in solid tumor patients. The complete remission levels were measured at 2–6 months from the initial diagnosis, at which time the patient was tumor free by all available clinical criteria. The TNF levels of the 130 healthy subjects are presented as reference values (Fig. 2). If the normal TNF level is defined as the 90% range of these values, the upper limit of normal TNF in serum is 40 ng/liter.

Fig. 2. Tumor necrosis factor α levels in serum of children with newly diagnosed malignant disease (n = 32), values of the same patients at complete remission status (n = 29), and values of healthy normal subjects (n = 130). P < 0.01 between diagnosis (DG) and complete remission (CR) of malignant disease. The lower limit of detection is 10, and the defined upper limit of normal is 40.

In children with ALL, the serum TNF concentration at diagnosis did not correlate with the total WBC count, age, or the ALL risk categories used. The follow-up of serum TNF levels in our ALL patients revealed a significant decrease of serum TNF during the first 4 weeks of therapy (Fig. 3). From week 4 to week 12, serum TNF remained relatively stable. Thereafter, it further decreased although it was still above the detection limit (Fig. 3).

Seven children with solid tumors received preoperative chemotherapy for 1–5 months. A clinical antitumor response correlated with decreasing serum TNF concentrations in 6 children, in 4 of these down to undetectable levels, while the primary tumor (osteosarcoma, neuroblastoma, Wilms' tumor, Askin tumor) was still present. One patient had poor response to chemotherapy combined with infected hematoma in the tumor, and his serum TNF level did not drop until after surgery.

DISCUSSION

Our data indicate that children with newly diagnosed malignant disease do have elevated serum TNF levels. This was especially the case with childhood ALL; 16 of 17 patients had elevated serum TNF at diagnosis (Fig. 1). On the other hand, when the patients achieved complete remission status, the serum TNF levels also concurrently normalized (Fig. 2).

The laboratory methods and the corresponding detection levels of TNF differ in the works published. The bioassay based on the lipoprotein lipase inhibition by TNF is relatively insensitive. In the enzyme-linked immunosorbent assay method, the TNF detection limit is reported as 45 ng/liter. The radioimmunoassay method used in the present study has a detection limit of 10 ng/liter (39). The immunological enzyme-linked immunosorbent assay and radioimmunoassay methods may not necessarily measure the intact functional TNF molecule. Even if we, in part, are measuring nonfunctional TNF, the assay still reveals a clear difference between untreated malignancy and normal status (P < 0.01; Fig. 2).

Our finding is in accord with the fact that elevated TNF values also have been demonstrated in adult patients with malignant disease (21–23, 38). Comparison to data using exactly the same laboratory method (38) reveals that the range of serum TNF in neoplastic disease is similar in adults and in children. It seems that endogenous TNF production indeed occurs in many patients with overt malignant disease.

The antitumor responses obtained in phase I clinical trials with exogenous recombinant TNF have been poor (19, 24–30), and there are many known and unknown problems involved in the clinical therapeutic use of TNF (40). Some human tumors that produce TNF in vitro appear resistant to the antitumor effects of TNF (41, 42). It also seems that the antitumor properties of TNF appear to require high concentrations and maximum tolerated doses (40). The serum TNF levels we and others (38) have measured in cancer patients may be more than 10 times lower than the concentrations required for observable therapeutic responses (25). This difference is even greater if we are not measuring the intact or functional TNF molecule.

The role of increased TNF production in malignancy is so far unclear. The question needs to be resolved whether this is part of the process of tumorigenesis itself or a way of host
response toward malignant disease. TNF has also been shown to be identical with cachectin (43). However, the role of this molecule in wasting, cachexia of cancer, and chronic disease remains to be determined. In our patients high serum TNF level at diagnosis was not correlated with a low relative weight, the best available estimate for a possible weight loss prior to diagnosis. Nevertheless, TNF is not specific for malignant disease alone; serum TNF ranges highest in bacterial infections (38). Even if TNF turns out to be similar to an acute phase reactant or a nonspecific indicator of disease, it may potentially be of value in the diagnostic work-up of new patients with suspected malignancy.

The serum TNF levels decreased into the reference range when the patients achieved complete remission. We, therefore, speculate that serum TNF levels may be of potential value in the follow-up of patients with neoplastic disease. In the children with ALL, the TNF levels dropped rather quickly, within 2–4 weeks (Fig. 3), which may also reflect the inhibition of TNF production by glucocorticoids; prednisone was used in the ALL induction regimens. In patients with pediatric solid tumors on neoadjuvant chemotherapy not including prednisone, a good clinical tumor response correlated with decrease of the TNF levels before operative removal of the primary tumor, consistent with the hypothesis that an inactive, involving, and/or necrotic tumor may not produce TNF or stimulate TNF production.

We have so far been unable to demonstrate any prognostic significance of the TNF levels at diagnosis in our material. In children with ALL, the serum TNF concentrations did not correlate with the initial leukocyte count or with the risk categories. Our follow-up is too short to reveal any changes in serum TNF before or at recurrent disease.

In conclusion, serum TNF levels are in general elevated in the follow-up of patients with neoplastic disease. In the children with ALL, the TNF levels dropped rather quickly, within 2-4 weeks (Fig. 3), which may also reflect the inhibition of TNF production by glucocorticoids; prednisone was used in the ALL induction regimens. In patients with pediatric solid tumors on neoadjuvant chemotherapy not including prednisone, a good clinical tumor response correlated with decrease of the TNF levels before operative removal of the primary tumor, consistent with the hypothesis that an inactive, involving, and/or necrotic tumor may not produce TNF or stimulate TNF production.

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