A Tumor Necrosis Factor-binding Protein Purified to Homogeneity from Human Urine Protects Cells from Tumor Necrosis Factor Toxicity*

Hartmut Engelmann, Dan Aderka, Menachem Rubinstein, Dalia Rotman, and David Wallach

From the Department of Virology, The Weizmann Institute of Science, Rehovot, Israel

Unfractionated preparations of the proteins of human urine provided protection against the in vitro cytocidal effect of tumor necrosis factor (TNF).

In certain cells, the proteins decreased expression of the receptors for TNF in a temperature-dependent way.

In all cells examined, the proteins were found to interfere also with the binding of both TNF and interleukin-1 when applied directly into the binding assays. That effect could be observed in the cold, suggesting that it was independent of cellular metabolism.

A protein which protects cells against the cytotoxicity of TNF was purified from human urine by chromatography on CM-Sepharose followed by high performance liquid chromatography on Mono Q and Mono S columns and reversed phase high performance liquid chromatography. This protein is a very minor constituent of normal urine, with an apparent molecular weight of about 27,000 in sodium dodecyl sulfate-polyacrylamide gel electrophoresis under both reducing and nonreducing conditions. Homogeneity of the purified protein was confirmed by microsequence analysis which revealed a single N-terminal sequence: Asp-Ser-Val-Cys-Pro-.

The protein protected cells from TNF toxicity at concentrations of a few nanograms per ml and interfered with the binding of both TNF-α and TNF-β to cells, when applied simultaneously with the cytokines. However, unlike crude preparations of the urinary proteins, the purified protein did not induce in cells a decrease in ability to bind TNF nor did it interfere with the binding of interleukin-1 to its receptor. Direct, specific binding to the protein of TNF-α and, to a lesser extent, also TNF-β, but not of interleukin-1 nor interferon-γ could be demonstrated. It is suggested that this protein blocks the function of TNF by competing for TNF with the TNF receptor and not by interacting with the target cell.

Tumor necrosis factor (TNF) is outstanding among the various mediators of immune defense in the extent to which it may cause harm to the host. Although it is effectively protective against various pathogens, this cytokine also has a mediating role in the pathological manifestations of diseases, including those caused by these very pathogens against which TNF can protect (for review, see Refs. 1 and 2). Exploring ways for suppressing the formation of TNF and antagonizing its destructive potential seems, therefore, of just as much practical importance as defining ways to take advantage of the beneficial effects of TNF in therapy.

Human urine has been shown to contain proteins which can interfere with the function of interleukin-1 (IL-1) (3–5). In view of the marked similarity in the physiological function of TNF and IL-1, we have posed the question whether proteins found in urine can also suppress the activity of TNF. We report here the purification to homogeneity and initial characterization of a protein which is present in human urine in minute amounts. This protein binds TNF, thus preventing its interaction with the TNF receptors and blocking its activity. It does not interfere with the binding of IL-1 to cells and differs in this, as well as in some other characteristics, from uromodulin, a urinary protein which suppresses the function of IL-1 (5–7) and, according to a recent study (7), also binds TNF with a high affinity, although it is apparently unable to interfere with its function. During preparation of this manuscript a study by Seckinger et al. (8) was published, describing a urinary antagonist to TNF which may be identical to the one described here.

MATERIALS AND METHODS

Cells

Murine A9 cells (9) and human foreskin fibroblasts, FS11 (established in our laboratory by Dr. D. Rotman), were cultured in Dulbecco's modified Eagle's minimal essential medium containing 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 μg/ml amphotericin B. The media were supplemented with 10% newborn calf serum for the A9 cells and with 10% fetal calf serum for the FS11 cells.

Cytokines

Recombinant human TNF-α (rhuTNF-α, 6×10^8 units/mg protein), recombinant murine TNF-α (rmTNF-α, 2.8×10^7 units/mg protein), and recombinant human TNF-β (rhuTNF-β, lymphotoxin, 1.2×10^6 units/mg protein) were kindly provided by Dr. G. Adelf, Boerhinger Institute, Vienna, Austria. Recombinant human IL-1α (rhIL-1α, 2.5×10^6 units/mg protein), consisting of the 154 carboxyl

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§ Holder of the Maurice and Edna Weiss Chair in Interferon Research.

† To whom correspondence and reprint requests should be addressed.

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terminal amino acids of the 271-amino acid human IL-1 precursor, was a gift of Drs. A. Stern and P. T. Lon nedico (Hoffmann La Roche, Nutley, NJ). Recombinant human interferon-γ (rIFN-γ) (5 × 10⁷ units/mg protein) was provided by Dr. D. Novick of our laboratory.

**Radiolabeling of Cytokines**

All cytokines were labeled with ¹²⁵I by the chloramine-T method, as previously described (10). The amounts of incorporated label in the preparations of cytokines used in this study were as follows: 112 μCi/μg protein for rmuTNF-α, 126 μCi/μg protein for rhuTNF-α, 113 μCi/μg protein for rhuTNF-β, and 159 μCi/μg protein for rIL-1-α.

The purified TNF-binding protein (TBP) was labeled by the same method to a specific activity of 238 μCi/μg protein.

**Assays for the TNF-binding Protein**

Quantitation of the Protective Effect of the TBP, against TNF Cytotoxicity—Mouse A9 cells were seeded in 96-well microtiter plates at a density of 15,000–20,000 cells/well. Urinary protein samples were applied, about 24 h later, together with cycloheximide (50 μg/ml) and rhuTNF-α (5 units/ml), and the cells were further incubated at 37 °C for 14 h. Cell viability was then quantitated by the neutral red uptake assay (11). For maximal sensitivity, the test was initiated when the A9 cells were just about to reach confluence. One unit of protective activity was defined as the amount of TNF-binding protein in whose presence the number of cells remaining viable, under the conditions of the assay, was doubled. The morphology of the A9 cells when protected from TNF toxicity by TBP is shown in Fig. 1.

Quantitation of the Binding of Cytokines to Cells and Its Decrease by Proteins of the Urine—A9 and FSII cells were seeded into 15-mm tissue culture plates at a density of 2.5 × 10⁴ cells/well. After 24 h incubation at 37 °C in 5% CO₂ atmosphere, the plates were transferred to ice, the growth medium was removed, and the radiolabeled cytokines (10⁵ cpm/plate, counting efficiency 50%) were introduced in 150 μl of ice-cold phosphate-buffered saline (PBS) (140 mM NaCl, 1.5 mM KH₂PO₄, 8 mM Na₂HPO₄, 2.7 mM KCl, 0.5 mM CaCl₂, 0.9 mM MgCl₂ containing 0.5% bovine serum albumin and 0.1% NaN₃ (PBS/BSA)). Following incubation for 2 h on ice, the cells were rinsed twice with ice-cold PBS/BSA and detached with Ca²⁺- and Mg²⁺-free PBS containing 5 mM EDTA. The cell-associated radioactivity was determined using a γ-counter. Non-specific binding of the cytokines, determined by adding 1000-fold excess of unlabeled cytokine, was subtracted from all values. Effects of urinary proteins on the binding of cytokines were examined by either applying the proteins directly into the binding assay or pretreating the cells by the proteins, for various durations, either at 37 °C (in growth medium) or at 4 °C (in PBS/BSA) and then removing the proteins prior to the quantitation of cytokine binding. Samples of the proteins were tested in duplicates.

**Solid Phase Assay for the Binding of Various Cytokines to the Purified TNF-binding Protein—PVC 96-well radioimmunoassay plates (Dynatech 1-220-25) were coated with rhuTNF-α, rhuTNF-β, rIL-1α, or rIFN-γ by incubation, for 12 h at 37 °C, with solutions of 5 μg/ml of the pure cytokines in PBS containing 0.02% NaN₃. The wells were then rinsed and incubated further, for 8 h at 4 °C, with PBS containing 0.5% BSA, 0.02% NaN₃, and 0.05% Tween-20 (blocking solution). Samples of radiolabeled TBP (10⁵ cpm in 50 μl of blocking solution) were then applied, either alone or in the presence of various cytokines or excess unlabeled TBP, and the plates were incubated for 12 h at 4 °C. They were then rinsed three times with blocking solution. The counts of the material which remained bound to the PVC plates were determined using a γ-counter.

**Purification of the TNF-binding Protein**

Concentration of the Crude Urinary Proteins (CUP)—Urine from healthy male donors was processed in pools of 300 liters. The urine was filtered on a Millipore HVLP membrane (pore size, 0.5 μm) and using a Pellicon cassette system. The filtrate was concentrated by tangential ultrafiltration to a final volume of 750 ml, with the aid of a PTGC Millipore membrane having a molecular weight cut off at 10,000. The concentrate was dialyzed against PBS containing 0.02% NaN₃ and 1 mM benzamidine (Sigma), divided into portions, and frozen.

**Chromatography on CM-Sepharose—**A CM-Sepharose (Pharmacia, Uppsala, Sweden) cation exchange column (2.7 × 10 cm) was equilibrated with 1 M NaCl, 10 mM citric acid, pH 5.0, containing 0.02% NaN₃ (buffer C) and equilibrated with 10 mM citric acid (pH 5) containing 0.02% NaN₃ (buffer A). The concentrated of urinary proteins was dialyzed against buffer A and centrifuged for 15 min at 8000 × g at 4 °C. The supernatant was applied to the column at a flow rate of 2 ml/min. The column was washed with 1500 ml of buffer A and eluted with 250 ml of a solution containing 200 mM NaCl, 10 mM citric acid (pH 5.0), and 0.02% NaN₃ (buffer B). A second step of elution was performed with 150 ml of buffer C. Fractions of 50 ml were collected and tested for biological activity, and their protein concentration was determined.

**Cation Exchange HPLC—**The active fractions eluted from the CM-Sepharose column were pooled, dialyzed against buffer A, and applied on a Mono S HR 5 × 50-mm column (Pharmacia). The column was washed at a flow rate of 0.5 ml/min until all unbound proteins were removed. The bound proteins were eluted with a linear NaCl gradient (0–350 mM NaCl in buffer A). The gradient was run for 40 min at a flow rate of 0.5 ml/min. The column was then washed for 10 min in buffer D (350 mM NaCl in buffer A), and further with buffer C. Fractions of 0.5 ml were collected and examined for a protective effect against TNF cytotoxicity, and their protein concentration was determined.

**Anion Exchange HPLC—**A fast protein liquid chromatography Mono Q 5 × 50-mm anion exchange column was equilibrated with 5 mM sodium borate (pH 9.0) containing 0.02% NaN₃ (buffer E). The active fractions eluted from the Mono Q column were pooled, dialyzed against buffer E, and loaded on the Mono Q column. The column was washed with buffer E until all unbound proteins were removed. The bound proteins were eluted at a flow rate of 0.5 ml/min, with a 300 mM NaCl linear gradient from 0 to 600 mM NaCl in buffer E followed by a 30-min linear gradient from 60 to 300 mM NaCl in buffer E. The column was then washed for 10 min with 300 mM NaCl in buffer E and subsequently for 4 min with 1 M NaCl in...
buffer E. Fractions of 0.5 ml were collected and examined as above.

**Reversed Phase HPLC**—The reversed phase HPLC column aqua-
apore RP300 (4.6 × 30 mm, Brownlee Labs), was prewashed with 0.3% aqueous trifluoroacetic acid (buffer F). The active fractions from the Mono Q column were pooled and loaded on the column. The column was washed with buffer E at a flow rate of 0.5 ml/min until all unbound material was removed; it was then eluted at a flow rate of 0.5 ml/min with a 0–20% linear gradient of acetonitrile in buffer F for 5 min followed by a 20–50% linear gradient of acetonitrile in buffer F for 60 min, and finally a 50–80% linear gradient of acetonitrile in buffer F for 5 min. The column was then washed with 80% acetonitrile in buffer F for 15 min. Fractions of 0.5 ml were collected and assayed as indicated.

**SDS-Polyacrylamide Gel Electrophoresis**—SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on 15% acrylamide gels was performed by the method of Laemmli (12) using the Bio-Rad Minigel device (thickness of gel, 0.5 mm). Proteins in the gel were visualized by silver staining (13).

**N-terminal Sequence Analysis**—Samples of the purified TBP were subjected to N-terminal sequence analysis on a pulsed liquid gas phase protein microsequencer (Model 475A Applied Biosystems Inc., Foster City, CA).

**Protein Determination**—Protein concentrations were determined by the fluorescamine method (14), using crystalline bovine serum albumin as a standard.

**RESULTS**

**Effects of Unfractionated Preparations of the Urinary Proteins on TNF Function**—Unfractionated preparations of the proteins of human urine had a marked, concentration-dependent, protective effect against the cytotoxicity of TNF (Fig. 2A, and see also Fig. 1).

To explore the mechanisms which underly this protection, we examined the effect of the proteins on the binding of TNF to its receptors. This test was carried out in several different ways: (a) pretreating the cells with the urine proteins at 4 °C, prior to the quantitation of TNF binding, to find out if these proteins block the TNF receptors; (b) pretreating the cells with the proteins at 37 °C, to see if there are components in the urine which down-regulate the TNF receptors; (c) supplying the proteins, simultaneously with TNF, directly to the TNF binding assay mixture, to find out if constituents of the urine interfere with the binding of TNF by an effect on TNF itself.

Pretreatment of human foreskin fibroblasts at 4 °C with the crude urinary proteins (CUP) had no effect on subsequent binding of TNF in the absence of the CUP (Fig. 3, left, top panel, 4 °C). However, after treatment with CUP at 37 °C, the cells exhibited a marked decrease in ability to bind TNF. This induced effect was rapid and transient, reaching a maximum within an hour of application of the proteins and then gradually decreasing (Fig. 3, left, top panel). A similar induced decrease in TNF binding has been reported in certain cells following treatment with IL-1 (15). Indeed, treating the human fibroblasts with IL-1, resulted in a decrease in TNF binding displaying kinetics similar to the kinetics of the effect induced by CUP (Fig. 3, left, middle panel). Murine A9 cells treated with CUP did not exhibit any such induced decrease in binding of TNF (not shown).

In addition, the CUP had a marked inhibitory effect on TNF binding when applied directly into the TNF binding
assay (Fig. 3, right, top panel). The effect occurred also at 4 °C and could not be increased further by preincubating TNF with the CUP at 37 °C (Fig. 2B). It could be observed in all cells examined, including the human foreskin fibroblasts (Fig. 2) and murine A9 cells (Table I). The CUP also interfered with the binding of TNF-β (lymphocytes) and IL-1 to cells, when applied simultaneously with the cytokines (Table I).

Several trivial causes for the effects of urinary proteins on the activity of TNF seemed to be excluded by the above observations. The presence of TNF itself in the urine would have resulted in interference by the urinary proteins with the binding of radiolabeled TNF and, in certain cells, also in induction of resistance to TNF toxicity (16). However this possibility seemed to be excluded by the fact that the urinary proteins did not interfere with the binding of TNF when applied to cells in the cold, prior to the application of TNF. Were TNF present in a free form in the urine, it would be expected to bind to the TNF receptors in such a pretreatment and thus block the binding of subsequently applied radiolabeled TNF. The presence of biologically active TNF in the CUP seemed to be excluded also by the fact that these preparations did not have any toxic effect on cells, not even when applied in the presence of cycloheximide, which sensitized cells to TNF toxicity (data not shown).

IL-1 has also been shown to increase, in certain cells, resistance to the toxicity of TNF, as well as a decrease in TNF binding (15, 17). However, unlike the urinary proteins, IL-1 does not interfere with TNF binding when applied to cells simultaneously with TNF, in the cold (Fig. 3, right, middle panel; see also Ref. 15) and, therefore, even if present in the urine, cannot account for that effect of CUP.

We also considered the possibility that the decrease in TNF binding and activity was due to degradation of TNF by some proteases known to be present in the urine. The fact that incubation of TNF with the urine proteins at 37 °C for 2 h prior to their application to the cells did not enhance the interference of the CUP with TNF binding (Fig. 2B), testifies that neither proteolytic degradation nor any other enzymic modification of TNF is involved in this effect. Furthermore, analysis by SDS-PAGE of the molecular size of TNF after its incubation with the urinary proteins revealed no signs of such degradation (not shown). Several agents known to block protease activity, N-ethylmaleimide, N- p-tosyl-L-lysine chloromethyl ketone, benzamidine, iodoacetamide, phenylmethanesulfonyl fluoride (all tested at a concentration of 1 mM), leupeptin (at 1 μg/ml), and aprotinin (at 0.5 unit/ml), did not interfere with the inhibition of TNF binding by the CUP (data not shown).

Involvement of antibodies in the effect of CUP seemed unlikely since, normally, urine does not contain antibodies. Furthermore, the molecular size of the proteins which mediate the effect on TNF activity, as estimated by size exclusion chromatography (see below), is clearly lower than that of immunoglobulins.

Purification and Initial Characterization of the Urinary TNF-binding Protein—A bioassay for the urinary protein(s) which mediate a protective effect against TNF cytotoxicity was established (see “Materials and Methods”); it then was used for the detection of these protein(s) throughout various fractionation steps.

Several chromatographic approaches for purification of the protein(s) were attempted. In size exclusion chromatography (on an Ultragel AcA44 column equilibrated with phosphate-buffered saline) only poor resolution between the protein(s) which interfere with TNF activity and other urinary proteins could be observed. Under these conditions, the activity fractionated together with the majority of the protein mass, peaking at an apparent molecular size of about 50,000–70,000. On the other hand, in isoelectric focusing, some enrichment of the proteins could be obtained. Consistent with prior observations, much of the protein mass of urine was found to be rather acidic, with an apparent isoelectric point lower than 5.0. Yet the isoelectric point of the protein(s) which protect cells from TNF toxicity was found to be close to 6.0 (data not shown). Fractionation by isoelectrofocusing for initial enrichment of the protein seemed impractical since only limited amounts of protein can be applied at a time. However, the pattern of isoelectric points revealed by that procedure indicated that enrichment of the protective protein on the basis of its charge properties should be possible.

As a first step, the urinary proteins were fractionated at pH 5.0 on a carboxymethyl-Sepharose column. Consistent with their acidity, most of the proteins did not bind to the resin under those conditions. However, the inhibitor of TNF activity was bound effectively to the resin at that step (about 80% of the applied activity), and most of it could be eluted from the column, together with about 1% of the initially applied protein, by increasing the ionic strength by 0.2 M NaCl. Increasing the ionic strength further (1 M NaCl) did not result in elution of any additional activity.

In the second purification step, the proteins were fractionated on a cation exchange HPLC column. The active protein was eluted at about 180–220 mM NaCl (Fig. 4). Peak fractions

![FIG. 4. Mono S cation exchange HPLC of TBP. The column was equilibrated with 10 mM citric acid (pH 5.0), 0.02% NaN3. Urinary proteins, enriched for TBP activity by fractionation on CM-Sepharose, were applied and then eluted with NaCl gradient (---). The effluent was monitored for absorbance at 280 nm (——) and for bioactivity (■).](image-url)
were pooled and subjected to further purification on an anion exchange HPLC column, from which the TNF inhibitory activity was eluted at a salt concentration of about 40 mM (Fig. 5).

The final fractionation step was on an Aquapore RP 300 reversed phase HPLC column. Proteins were eluted from the column by applying a gradient of acetonitrile. The active protein was found to elute as a distinct protein peak, at about 27% acetonitrile (Fig. 6). The molecular size was independent of whether or not the analysis was performed in the presence of a reducing agent (β-mercaptoethanol). Homogeneity of the purified protein was further confirmed by N-terminal microsequence analysis. A single sequence, Asp-Ser-Val-Cys-Pro-, was obtained in the analysis at a high yield (initial yield, 67%).

Both from the low amounts of the protein, recovered in the purification, and from comparison with the protein pattern in the crude preparation, as revealed by SDS-PAGE, it is clear that the factor is a very minor constituent of urine. In the urine preparation, used for the fractionation whose results are presented in Table II, the protein constituted 10⁻³⁻¹⁰⁻⁴% of the total protein. Specific activity of the purified protein was about 50,000-fold higher than that of the CUP.

Like the crude preparations of the urinary proteins, the purified protein interfered with the binding of TNF-α (of both human and mouse origin), as well as with huTNF-β when applied to cells simultaneously with these cytokines (Table I and Fig. 3, right, bottom panel). However, it did not interfere, to any measurable extent, with the binding of IL-1 to its receptor (Table I) nor did it induce in cells, pretreated with the protein, a decrease in ability to bind TNF (Fig. 3, left, bottom panel). The latter two effects of crude preparations of the urinary proteins therefore seem to be mediated by some other constituents of the urine.

The binding properties of the urinary protein were explored using radiolabeled preparations of the purified protein. As shown in Table III, the labeled protein was found to bind to immobilized TNF-α and, to a much lesser extent, to TNF-β. This binding could be competed by TNF-α and TNF-β and by excess of the unlabeled urinary protein. IL-1 (as well as IFN-γ) did not bind the urinary protein, nor did it compete for the binding of the protein to TNF.

### FIG. 6. Reversed phase HPLC of TBP. The proteins enriched for TBP activity on the Mono Q column were applied to an Aquapore RP300 column. Elution was performed with a gradient of acetonitrile in 0.3% aqueous trifluoroacetic acid (---). Fractions were examined for bioactivity (■), and protein (———) content. Shown in inset is SDS-PAGE analysis of proteins in the absence of reducing agents (A, B), or in the presence of 15% β-mercaptoethanol (C). The pattern of proteins in the preparation of unfractionated urinary proteins prior to chromatography on CN-Sepharose (5 µg, lane A), is compared to the protein in fraction 21 of the elution from the HPLC RP300 column (0.1 µg, lanes B and C). The pattern of proteins in the preparation of unfractionated urinary proteins prior to chromatography on CN-Sepharose (5 µg, lane A), is compared to the protein in fraction 21 of the elution from the HPLC RP300 column (0.1 µg, lanes B and C), where only a single polypeptide band can be discerned. (The faint high molecular weight bands in C could be observed also in the absence of any added protein and seem to reflect the presence of some contaminants in the β-mercaptoethanol.)

### FIG. 5. Mono Q anion exchange HPLC of TBP. Active fractions eluting from the Mono S column were made up to 5 mM sodium borate (pH 9.0), 0.02% NaN₃, and applied to the Mono Q column. The proteins were then eluted with an NaCl gradient (——) and the fractions containing the TBP were pooled, based on quantitation of bioactivity (■) and protein (——).
crude urine against TNF toxicity, seems to function only by affecting the TNF molecules; it showed no direct effect on cells and was unable to interfere with the binding of IL-1 to its receptors.

The purified protein acts by binding the cytokine and thus competing for it with the TNF receptors. Although it does not bind IL-1, it does bind TNF-α and, with much lower effectiveness, also TNF-β (lymphotoxin). The specific nature of its interaction is further demonstrated by the inability of the protein to bind another cytokine-IFN-γ. Even though TNF-α and TNF-β share only partial structural homology (18), they compete for binding to the same cell surface receptor (19). The fact that they both bind to the urinary protein raises the possibility that this protein associates with that part of the cytokine molecule which is recognized by the receptor.

By its inability to interfere with the binding of IL-1 to cells, the TNF-binding protein (TBP) can be distinguished from uromodulin, a major glycoprotein of the urine, of greater molecular size (85,000), which was reported to have a high binding affinity to both IL-1 and TNF and to interfere with the function of IL-1, although not with that of TNF (5-7). The TBP is also clearly distinct from another antagonist to IL-1, shown recently to be present in urine, which interferes with IL-1 binding apparently by binding competitively to the IL-1 receptor, but it seems unable to block the function of TNF (4, 20, 21).

The presence of the above antagonists in urine probably accounts for the inhibitory effect of CUP on IL-1 binding. At the same time, the induced decrease in the ability of FS11 cells to bind TNF following treatment with CUP appears to be very similar to an effect of IL-1 itself. IL-1 induces a decrease in binding of TNF, which apparently reflects a decrease in expression of the TNF receptors. In FS11 fibroblasts, although not in some other cells (15), this decrease is transient and its kinetics resembles the one seen with urinary proteins. Some evidence for the presence of IL-1 in the urine has been reported (4, 22). Whether indeed IL-1, if present in the unfractionated preparations of the urine, can mediate the effect in spite of its coexistence with proteins which block its activity, or whether it is some other constituent(s) of the urine which functions in our test system similarly to IL-1, remains to be determined.

The protein whose purification is described in this study is present in the urine in minute amounts, constituting about 10^-3-10^-4% of the total protein mass. It would not have been detected save for the fact that it is very active. Indeed, it can be calculated that a unit of protective activity (the activity resulting in a 2-fold increase in the number of cells which remain viable after challenge with TNF) is mediated by the pure protein at a concentration of about 2 ng/ml, which is equivalent to about 6.10^-11 M protein.

The physiological role of the protein remains to be elucidated. It is tempting to speculate that, just as in our in vitro experimental system, this protein functions in vivo as an antagonist to TNF. However, to test this hypothesis it will be necessary first to determine how this protein is formed and what results from its interaction with TNF under in vivo conditions. At present, one cannot rule out that this protein has a completely different in vivo role, perhaps even a converse one; it might prolong the exposure of the organism to endogenously produced TNF by binding the cytokine and then releasing it slowly in an active form.

There is some evidence both for the existence of mechanisms whereby the organism can protect itself from the potentially destructive effects of TNF and for enhanced expression of these mechanisms following exposure to TNF. Several studies have shown that vulnerability to certain deleterious effects of TNF and of bacterial components which can induce TNF production is markedly reduced following exposure of the organism to TNF itself or to IL-1 (23-26). If the protein described in the present study can indeed contribute to such protection, it is very likely that useful applications for it will be found, specifically as a therapeutic agent in those pathological situations where TNF can have detrimental effects.

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Addendum—Recently, we have determined the sequence of the 18 N-terminal amino acids of the TBP. A search through the NBRF protein data bank, release 18, failed to reveal significant homology to any of the other known protein sequences. In the 2nd International Conference on TNF and Related Cytokines held January 15-20, this year, I. Olsson from the University of Lund, Sweden, also reported on the purification of a urinary protein which binds TNF. Based on comparison of N-terminal amino acid sequences and of chromatographic properties, this protein appears identical to the one whose purification is described in the present study.2

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