Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 5188–5192, July 1990
Immunology

Characterization of a tumor necrosis factor α (TNF-α) inhibitor: Evidence of immunological cross-reactivity with the TNF receptor

PHILIPPE SECKINGER, JIAN-HUA ZHANG, BETTINA HAUPTMANN, AND JEAN-MICHEL DAYER*

Division of Immunology and Allergy (Hans Wilsdorf Laboratory), Department of Medicine, Hôpital Cantonal Universitaire, 1211 Geneva 4, Switzerland

Communicated by Jerome Gross, April 30, 1990 (received for review February 1990)

ABSTRACT Previous studies have shown that urine of febrile patients contains a tumor necrosis factor α inhibiting activity (TNF-α Inh) when tested in a cytotoxicity assay using the tumor necrosis factor α (TNF-α)-susceptible cell line L929. In the present study, we investigated the relationship between the TNF-α Inh and a potential soluble form of the receptor, as the former has been shown to block TNF-α activities by binding to the ligand. We demonstrate that human TNF-α is affected to a greater extent than is murine TNF-α. This species specificity of the inhibitor correlates with the binding studies of TNF receptor interactions already reported. We raised a polyclonal antibody to TNF-α Inh that neutralizes its activity and does not recognize TNF-α. Solubilized cross-linked ^{125}I-labeled TNF-α receptor complex could be immunoprecipitated by using either anti-TNF-α or anti-TNF-α Inh antibody, suggesting immunological cross-reactivity between the receptor and the inhibitor. By using fluorescein isothiocyanate-coupled TNF-α, it was possible to visualize by fluorescence-activated cell sorter analysis the TNF-α receptor on phytohemagglutinin/interleukin 2-activated T cells. A similar increase of immunofluorescence intensity of the activated T cells was observed by using anti-TNF-α Inh antibody revealed with a fluorescein isothiocyanate-coupled goat anti-rabbit IgG1 conjugate, suggesting that the TNF-α Inh is also expressed as a membrane protein. Taken together, our results suggest that the TNF-α Inh originally described might be a soluble form of the TNF receptor itself.

Tumor necrosis factor α (TNF-α), originally described as a monocyte product with antitumoral activity both *in vitro* and *in vivo*, is now reported to have multiple biological activities (1–3). TNF-α exerts its cellular responses by binding to high-affinity cell-surface receptors, which have been recently characterized (4, 5). Inhibitory activity toward TNF-α was originally detected in urine of febrile patients (6) and later in urine from normal donors as well as in plasma (7–10). In contrast to the inhibitors of interleukin 1 (IL-1) (11), this inhibitory activity to TNF-α appears to be homogeneous with respect to biochemical and biological characteristics.

In addition to the cytotoxicity inhibition reported by us and others (6–9), we demonstrated that the TNF-α inhibitor (TNF-α Inh) regulates TNF-α inflammatory and immunomodulatory properties by affecting TNF-α-induced prostaglandin E_2 production by dermal fibroblasts, class I, and interferon γ-induced HLA class II antigen expression on the Colo 205 human cell line (7, 8). The functions of various cytokines are also controlled by the regulation of their receptors. Since TNF-α Inh acts by binding to the ligand, we wondered whether the present inhibitor might be a soluble form of the TNF receptor itself, acting as a negative feedback mechanism. A similar phenomenon has already been reported for a soluble murine IL-4 receptor (12). Our data indicate that the TNF-α Inh has, as the TNF receptor, species specificity. A rabbit polyclonal antibody raised against the TNF-α Inh, which does not recognize TNF-α, immunoprecipitates the solubilized ^{125}I-labeled TNF (^{125}I-TNF) receptor complex of the U937 cell line. In addition, expression of TNF receptors on phytohemagglutinin (PHA)/IL-2-activated T cells fits with membrane expression of TNF-α Inh.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Reagents and Media. Phosphate-buffered saline, fetal calf serum, penicillin, streptomycin, glutamine, minimal essential medium, and RPMI 1640 medium were obtained from GIBCO. PHA was purchased from Wellcome. Recombinant human TNF-α (hTNF-α), recombinant murine (mTNF-α), and recombinant human IL-2 were produced in *Escherichia coli* (Biogen). Goat polyclonal antibody to hTNF-α was provided by R. Ulevitch (Scripps Clinic, La Jolla, CA). Rabbit antibody to anti-hIL-1β was obtained from Glaxo.

Cell Culture. The murine fibroblast L929 cell line was cultured as described (6). Human purified peripheral blood T cells (13) were first stimulated with PHA (5 μg/ml) in the presence of IL-2 (20 ng/ml) and then for 6 more days in the presence of IL-2 alone at the same concentration. All cultures were prepared at 37°C in 5% CO_2/95% air in a humidified atmosphere. TNF-α Inh bioassays were performed as described (11) by using various concentrations of hTNF-α (0.025–1.0 ng/ml) or mTNF-α (2.5–500 pg/ml).

TNF-α Inh Purification. TNF-α Inh was purified to homogeneity by sequentially using a TNF-α affinity column, Mono S cation-exchange, and reverse-phase chromatographies. The specific activity of TNF-α Inh is 1.38×10^6 units/mg. One unit of TNF-α Inh is defined as the amount of inhibitor that blocks 50% of TNF-α (0.2 ng/ml)-induced cytotoxicity. The percentage of TNF-α Inh is calculated by the formula

$$100 \times \left(\frac{OD_{(\text{actinomycin D+hTNF-}\alpha\text{+TNF-}\alpha\text{ Inh})} - OD_{(\text{actinomycin D+hTNF-}\alpha)}}{OD_{(\text{actinomycin D})} - OD_{(\text{actinomycin D+hTNF-}\alpha)}}\right). \quad [1]$$

Antibodies to TNF-α Inh. A 6-month-old rabbit (New Zealand White) was immunized with TNF-α Inh at a dose of ≈3 μg, injected as emulsion in complete Freund's adjuvant. Sequentially 3, 2, and 1 week later, the animal was injected with the same amount of TNF-α Inh in incomplete Freund's adjuvant. Blood was withdrawn 1 week after the two last injections and the serum was tested for antibody to purified TNF-α Inh. IgG was purified by using a protein G affinity column (Pharmacia) as recommended by the manufacturer.

Western Blotting Analysis. Proteins to be tested were applied to a polyacrylamide gel in the presence of SDS, run as described by Laemmli (14), and then blotted electrophoretically onto a nitrocellulose membrane (Schleicher & Schüll).

Abbreviations: TNF-α, tumor necrosis factor α; h-, human; m-, murine; TNF-α Inh, TNF-α inhibitor; FITC, fluorescein isothiocyanate; IL, interleukin; PHA, phytohemagglutinin; EGS, ethyleneglycolbis(succinimidylsuccinate).

*To whom reprint requests should be addressed.

The transferred proteins were incubated for 3 hr in 10 mM Tris·HCl (pH 7.4) containing 150 mM NaCl and 3% bovine serum albumin (Sigma) and were then rinsed in 10 mM Tris·HCl (pH 7.4) containing 150 mM NaCl and 0.1% Triton X-100. The nitrocellulose membrane was then incubated overnight with the antibody to be tested, followed by incubation for 1 hr with ^{125}I-labeled protein G (Amersham). After washing off unbound material, the nitrocellulose sheets were exposed for autoradiography.

Immunoprecipitation Analysis. Cross-linking of ^{125}I-TNF-α to cell-surface receptor was carried out as described by using ethyleneglycolbis(succinimidylsuccinate) (EGS) (Sigma) (5). The solubilized receptor was immunoprecipitated according to the method of Stauber *et al.* (5) by using either goat anti-TNF-α, rabbit anti-TNF-α Inh, or rabbit anti-hIL-1β at a final dilution of 1:100. The ligand–receptor and the immunoprecipitated complexes were subjected to SDS/PAGE run under reducing conditions (5, 14).

Fluorescence-Activated Cell Sorter Analysis. Fluorescein isothiocyanate (FITC) conjugation of TNF-α was carried out as described for IL-1α (15, 16) and immunofluorescence staining of cell-surface TNF receptor of PHA/IL-2-cultured T cells was carried out as described by Owen-Schaub *et al.* (16). Membrane expression of TNF-α Inh was also measured on PHA/IL-2-activated T cells by using protein G-purified IgG anti-TNF-α Inh. For the indirect immunofluorescence staining of cell surface, a FITC-coupled goat anti-rabbit IgG_1 (Coulter) was used as the second antibody at a dilution of 1:40. Flow cytometric analysis was performed with an EPICS V cell sorter (Coulter).

RESULTS

Specificity of TNF-α Inh for mTNF-α and hTNF-α. Mature mTNF-α and hTNF-α are highly conserved hormones since 79% identity is observed on the primary structure of both proteins (17, 18). We investigated whether the TNF-α Inh exhibits some specificity for either hTNF-α or mTNF-α. Addition of increasing concentrations of either mTNF-α or hTNF-α to actinomycin D-treated L929 cells resulted in a dose-dependent cytotoxicity (Fig. 1). However, in our L929 assay, the specific activity of mTNF-α is 1 logarithm higher than that of hTNF-α, since half-maximal cytotoxicity was obtained at concentrations ranging between 2, 5, and 10 pg/ml, whereas hTNF-α half-maximal cytotoxicity was obtained at concentrations of ≈50 pg/ml. Addition of TNF-α Inh at 5 units/ml resulted in the inhibition of cytotoxicity induced by both mTNF-α and hTNF-α. At 5 units/ml, TNF-α Inh reversed cytotoxicity by ≈100% and ≈75% when hTNF-α concentrations were 50 and 500 pg/ml, respectively. Contrasting with the effect observed on hTNF, 5 units of TNF-α Inh per ml reversed cytotoxicity by ≈80% and ≈20% when induced by 50 and 500 pg of mTNF-α per ml, respectively. These data indicate that the TNF-α Inh presents species specificity as it affects hTNF-α more than mTNF-α. Similarly, we found previously that hTNF-α is inhibited to a greater extent than lymphotoxin (7).

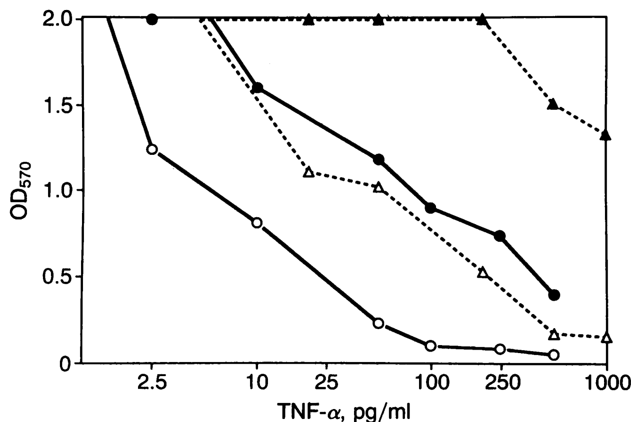

FIG. 1. Specificity of TNF-α Inh. Cell cytotoxicity was induced in either the absence (○) or the presence (●) of TNF-α Inh (5 units/ml) and increasing concentrations of mTNF-α. Cell cytotoxicity was induced in either the absence (△) or the presence (▲) of TNF-α Inh (5 units/ml) and increasing concentrations of hTNF-α. In both cases, cell cytotoxicity was measured as described and values represent means ± SE (n = 3) of cell lysis measured by dye uptake at 570 nm.

Characterization of a Rabbit Polyclonal Antibody to TNF-α Inh. Polyclonal antibodies to purified TNF-α Inh were raised to investigate the potential immunological cross-reactivity between the inhibitor and the TNF receptor. Therefore, a New Zealand White rabbit was injected as described. Serum samples from preimmune and immune animals were tested for the inhibition of TNF-α Inh activity, thereby restoring the biological activity of TNF-α in the standard L929 assay. Thus, 2, 5, and 20 units of TNF-α Inh per ml were preincubated for 1 hr at 37°C with various dilutions of serum and were tested against 0.2 ng of hTNF-α per ml. As shown in Fig. 2*A*, preincubation of TNF-α Inh with polyclonal antibodies neutralized the protective effect of TNF-α Inh. Fifty percent of TNF-α Inh activity was observed for serum dilutions of

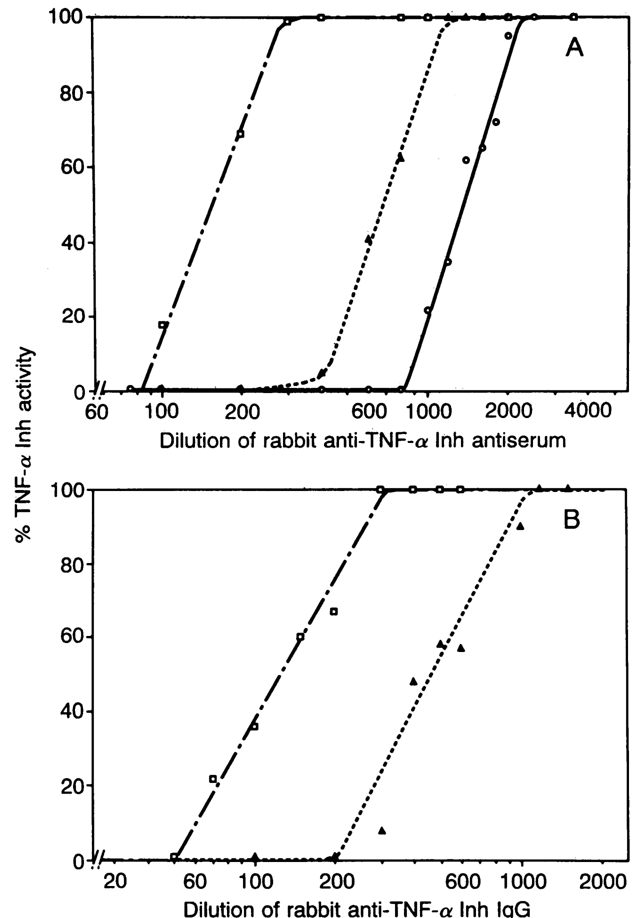

FIG. 2. Rabbit polyclonal antibodies neutralize TNF-α Inh activity. (*A*) Two (○), 5 (▲), and 20 (□) units of TNF-α Inh per ml were preincubated on cells for 1 hr at 37°C with various dilutions of anti-TNF-α Inh antiserum. hTNF-α (0.2 ng/ml) was added after preincubation. Cell cytotoxicity was measured as described. (*B*) Five (▲) and 20 (□) units of TNF-α Inh per ml were preincubated on cells for 1 hr at 37°C with various dilutions of protein G-purified IgG of anti-TNF-α Inh antiserum. hTNF-α (0.2 ng/ml) was added after preincubation. Cell cytotoxicity was measured as described.

1:1400, 1:680, and 1:160 for 2, 5, and 20 units/ml, respectively, suggesting that 1 unit of TNF-α Inh is neutralized by an antiserum dilution of $\approx$1:3000. When tested at the same dilution in the absence of hTNF-α, this antiserum was not capable of mimicking the monokine activity (data not shown). When preimmune serum from the same animal was tested, it failed both to block TNF-α Inh activity and to mimic TNF-α on L929 cells (data not shown). Similar results were obtained when protein G-purified IgG was used (Fig. 2*B*). Thus, 50% of TNF-α Inh activity was observed when the concentrations of proteins added were 90 and 350 μg/ml for 5 and 20 units/ml, respectively, so that 1 unit of TNF-α Inh is neutralized by the addition of $\approx$18 μg of protein G-purified IgG per ml. When preimmune protein G-purified IgG from the same animal was tested at the same protein concentrations, it neither blocked TNF-α Inh activity nor mimicked TNF-α on L929 cells (data not shown).

TNF-α is a trimeric molecule, each subunit of which consists of an antiparallel β sandwich (19). TNF-α Inh has been shown to bind TNF-α itself (7). It was therefore important to investigate whether there exists some homology(ies) in the structure of the two proteins allowing TNF-α Inh to behave as TNF-α subunit, resulting in an altered TNF trimer devoid of biological activity. Therefore, Western blotting of hTNF-α and TNF-α Inh was performed and reactivity of antibodies to hTNF-α or TNF-α Inh with SDS-treated hTNF-α and TNF-α Inh after electrophoresis was performed. As shown in Fig. 3 (lane C), polyclonal antibodies to TNF-α Inh produced a band in the expected region of 33 kDa. The same polyclonal antibody did not produce any band when hTNF-α was run on the gel (lane D). Similarly, polyclonal antibodies to hTNF-α produced a band in the expected region of 17 kDa (lane B). In contrast, the same polyclonal antibody did not produce any band when TNF-α Inh was run on the gel (lane A). These data indicate that the antibodies under investigation show reactivity with their substrate and that TNF-α and TNF-α Inh do not share immunologic cross-reactivity.

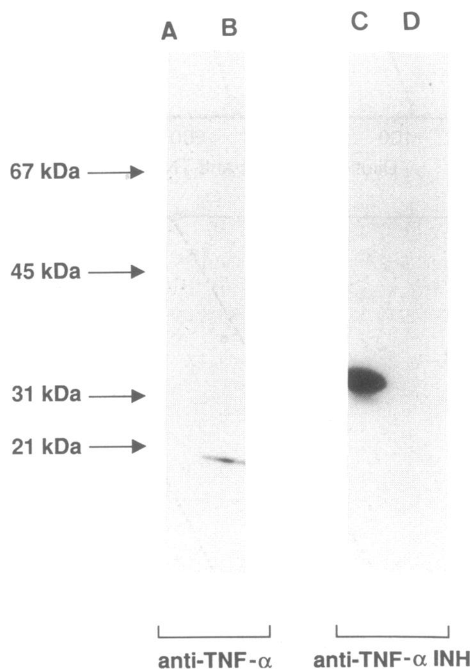

FIG. 3. Western blotting of hTNF-α and TNF-α Inh with the use of goat polyclonal antibodies to hTNF-α and rabbit antibodies to TNF-α Inh. hTNF (0.1 μg; lanes B and D) or TNF-α Inh (500 units; lanes A and C) was run on SDS/polyacrylamide gel and transferred to nitrocellulose. Lanes: A and B, incubated with goat antiserum against hTNF-α; C and D, incubated with rabbit antiserum against TNF-α Inh and revealed by using ^{125}I-labeled protein G (10^6 cpm per lane). After transfer, the membrane was autoradiographed for 72 hr.

Immunoprecipitation of TNF-α Receptor with Antibodies to TNF-α Inh. ^{125}I-TNF-α bound to cell surface was covalently cross-linked to the receptor by using EGS. The cross-linked receptor–ligand complex was solubilized with Triton X-100 (final concentration, 0.4%) and lyophilized, and an equivalent amount of 10^7 U937 cells was loaded and analyzed by SDS/PAGE. The autoradiogram of the gel shows a band at $\approx$95 kDa only when the cells are incubated with ^{125}I-TNF-α alone (Fig. 4, lane A). The smaller molecular mass bands represent monomeric and polymeric forms of ^{125}I-TNF-α (lane A). These results are in agreement with the data reported by Stauber *et al.* (5) on solubilized TNF receptor of U937 cells. The trimeric protein cross-link of TNF-α (51 kDa) was only visualized when the gel was autoradiographed for 2–3 days more.

We reasoned that if the TNF receptor shares some immunological cross-reactivity with the TNF-α Inh, then the cross-linked TNF receptor complex should be immunoprecipitated by either anti-TNF-α or anti-TNF-α Inh antibodies. Therefore, we analyzed on the same SDS/polyacrylamide gel both solubilized cross-linked receptor complex and its immunoprecipitation by either anti-TNF-α or anti-TNF-α Inh antibodies. The cross-linked ^{125}I-TNF-α ligand–receptor complex is recognized and immunoprecipitated by goat anti-TNF-α (Fig. 4, lane B) and rabbit anti-TNFα Inh (lane C). In

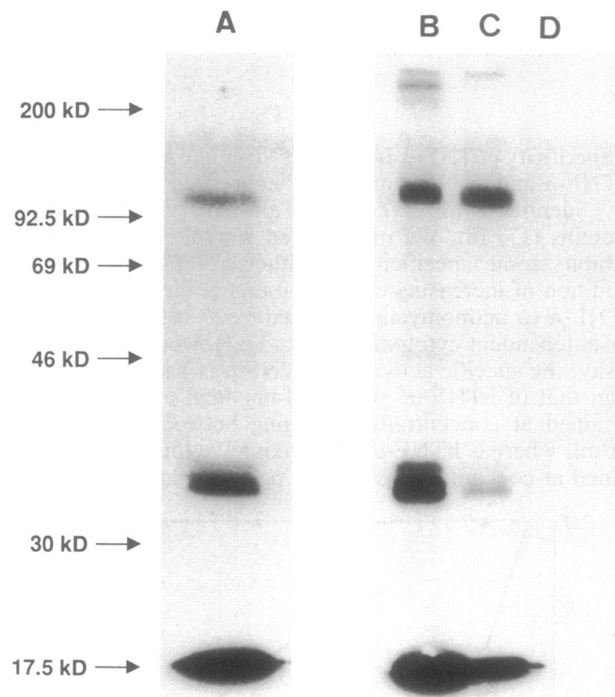

FIG. 4. SDS/PAGE analysis of receptor–ligand complex and its immunoprecipitation. ^{125}I-TNF-α bound to 10^7 U937 cells was treated with freshly prepared EGS at a final concentration of 0.1 mM for 40 min. The reaction was quenched, and cells were solubilized according to the method of Stauber *et al.* (5). Samples were resolved by SDS/PAGE and then visualized by autoradiography. Lane A, binding of U937 cells with ^{125}I-TNF-α. ^{125}I-TNF-α bound to 15×10^7 U937 cells was treated with freshly prepared EGS as in lane A. An identical amount of receptor–ligand complex solubilized with 5×10^7 U937 cells was incubated with either anti-TNF-α (lane B), anti-TNF-α Inh (lane C), or anti-hIL-1β (lane D) antiserum as described. The immune complexes were precipitated with protein A bound to Sepharose beads. The resulting pellets were extracted with SDS sample buffer, resolved on the same SDS/12% acrylamide gel as the solubilized ^{125}I-TNF-α receptor complex, and then visualized by autoradiography.

contrast, the ligand–receptor complex could not be immunoprecipitated by using a nonspecific anti-hIL-1β antiserum (lane D). The smaller molecular mass bands represent monomeric and polymeric forms of ^{125}I-TNF-α (lanes B and C).

Induction of TNF Receptor Expression on IL-2-Stimulated T Cells. To confirm our previous observation of immunological cross-reactivity between TNF receptor and TNF-α Inh, we investigated whether we could visualize the appearance of TNF receptors on IL-2-cultured T cells by using either FITC-coupled TNF-α or anti-TNF-α Inh revealed with a FITC-coupled goat anti-rabbit IgG1. Therefore, PHA/IL-2-activated T cells were cultured for 6 days in the presence of IL-2 alone, and TNF receptors were visualized by using a flow cytometric FITC-coupled TNF-α binding technique. Specific TNF-α binding was detected in IL-2-activated T cells and nonspecific binding was determined by addition of a 100-fold molar excess of unlabeled TNF-α (Fig. 5*A*). Moreover, control unstimulated cells expressed only a few TNF receptors. A similar pattern of immunofluorescence was obtained when purified IgG fractions from anti-TNF-α Inh antibodies, revealed by FITC-coupled goat anti-rabbit IgG1, were used for the immunofluorescence staining of cell surface (Fig. 5*B*). The cell number expressing specific staining is significantly increased when compared to the nonspecific staining obtained by using the preimmune serum at the same protein concentration. Control unstimulated cells express a slightly increased immunofluorescence pattern as compared to cells stained with preimmune serum (Fig. 5*C*). Taken together, these results suggest that the TNF receptor expression can be visualized by using either FITC-coupled TNF-α or anti-TNF-α Inh antibodies, confirming our previous result that the TNF receptor shows immunological cross-reactivity with the TNF-α Inh.

DISCUSSION

Recent data indicate that a soluble form(s) of receptor might be important for cytokine regulation (12). Since the TNF-α Inh under investigation acts by binding to the ligand, it is reasonable to hypothesize that, in fact, this inhibitor represents a soluble form of the TNF receptor. We found previously that the TNF-α Inh affects lymphotoxin only to a small extent when compared to hTNF-α (7). Recently, Stauber *et al.* (20) found that hTNF-α is $\approx$2-fold more potent than human lymphotoxin in displacing ^{125}I-lymphotoxin from its receptor. This difference could not be correlated to the specific activities of the two peptides because the specific activity of human lymphotoxin is 3-fold higher than that of hTNF-α. Both genes—h- and mTNF-α—have been cloned (17, 18), and previous studies have indicated that hTNF-α competes with mTNF-α for receptor binding on murine cells but that mTNF-α exhibited higher affinity to the murine receptor than hTNF-α (21, 22). Similarly, the TNF-α Inh is $\approx$10 times more efficient in affecting hTNF-α when compared to mTNF-α in the L929 assay of cytotoxicity. Taken together, these results suggest that, like the TNF receptor, the TNF-α Inh shows species specificity, as it affects hTNF-α more than mTNF-α, and it exhibits specificity for TNF-α versus lymphotoxin.

In light of the immunological cross-reactivity between TNF-α receptor and TNF-α Inh, these data are in agreement with the hypothesis that the inhibitor behaves as a soluble form of the TNF receptor. The structure of TNF-α has been found to be a trimer in solution by using diverse techniques such as analytical ultracentrifugation (23), cross-linking (24), or small x-ray scattering (25), and TNF-α binds also as a trimer to its receptor (26). It might, therefore, be possible that the trimeric structure of the polypeptide is altered by a TNF-α analogue. However, our results argue against this hypothesis since the TNF-α Inh shares no antigenicity with TNF-α as determined by Western blotting. The SDS/PAGE analysis of cross-linked TNF receptor complex reveals subunits of TNF-α bound to a monomeric 80-kDa receptor and confirms previous investigations on U937 cells (5). The TNF-α–receptor complex could be immunoprecipitated by using anti-TNF-α or anti-TNF-α Inh antiserum. The band of 95 kDa, corresponding to the cross-linked complex, was recovered by using both polyclonal antibodies A, whereas control anti-IL-1β remained ineffective. The bands at 17, 34, and 51 kDa correspond to monomers and polymers of ^{125}I-TNF-α, as already reported by Stauber *et al.* (5). These data indicate immunological cross-reactivity between TNF-α Inh and TNF receptor. Interestingly, urine has been proven to be a rich source of specific receptors for IL-6 and interferon γ (27), and the present inhibitor has also been purified from urine. More recently, the murine IL-4 receptor was cloned and its soluble form was found to block the lymphokine-induced biological activities (12). These findings support the hypothesis that soluble forms of receptors might be important for the regulation of hormone activity. Recently, two different receptors for TNF-α have been identified (28) and TNF binding proteins that might represent soluble forms of these receptors were found in urine independent of our study (29). To firmly establish immunological cross-reactivity of the TNF-α inhibitor with the TNF receptor, we examined the parallel immunofluorescence staining of the same cells with FITC-coupled TNF-α or with anti-TNF-α Inh. Several groups have reported that T cells cultured with either IL-2 alone or IL-2 in the presence of PHA express high numbers of TNF receptors (16, 30). We chose to culture T cells in the

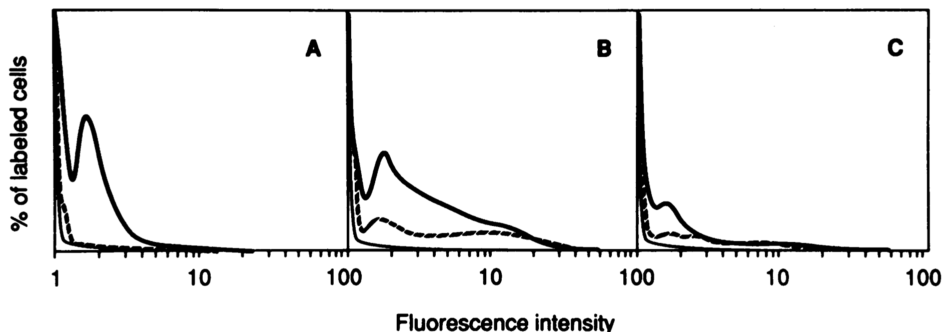

FIG. 5. Immunofluorescence staining of IL-2-stimulated peripheral T cells. (*A*) Fluorescence-activated cell sorter (FACS) analyses of TNF receptor expression measured by specific binding of FITC-coupled TNF-α. T cells were cultured in medium alone (---) or in PHA/IL-2 (——). Nonspecific binding was determined by addition of a 100-fold excess of unlabeled hTNF-α (——). (*B* and *C*) FACS analyses of membrane expression of TNF-α Inh. T cells were cultured in medium alone (*C*) or in PHA/IL-2 (*B*). In *B* and *C*, cells were stained either with anti-TNF-α Inh (——) or with preimmune anti-TNF-α Inh (---) and were revealed with FITC-coupled goat anti-rabbit IgG_1. Nonspecific immunofluorescence (——) was determined by using FITC-coupled goat anti-rabbit IgG_1 at a dilution of 1:40.

presence of PHA/IL-2 during the first 24 hr since they express 2- to 3-fold more TNF receptors than when cultured in the presence of IL-2 alone. Visualization of TNF receptors on T cells by using FITC-coupled TNF-α indicated that the TNF receptor was specifically labeled. Immunofluorescence staining of activated T cells by using polyclonal IgG raised against purified TNF-α Inh revealed a peak of fluorescence that is also observed when using FITC-coupled TNF-α. However, a population of cells expressing an increase of fluorescence intensity is revealed by the use of these polyclonal IgG anti-TNF-α Inh that is not seen with FITC-coupled TNF-α. These data suggest that anti-TNF-α Inh recognizes additional structures on the cell surface. Recently, Stauber *et al.* (20) were able to visualize two bands at molecular masses of 100 and 120 kDa when ^{125}I-lymphotoxin was cross-linked to its receptors and the latter was not seen when ^{125}I-TNF-α was used. Cross-linking of ^{125}I-lymphotoxin to its receptor and immunoprecipitation with the use of anti-TNF-α Inh would answer the question of whether the inhibitor also recognizes the 120-kDa structure. Alternatively, T cells represent a heterogeneous population, and it cannot be excluded that this represents TNF receptor expression of cellular subpopulations as its heterogeneity in regard to the molecular mass has been reported by several groups (31–33). This is less likely, however, because FITC-coupled TNF-α should bind its own receptor independently of the cellular subpopulation. Interestingly, the TNF receptor is slightly expressed on unstimulated T cells and this is in agreement with our immunofluorescence staining of the control cells (30).

In conclusion, our data suggest that the TNF-α Inh has specificity as it affects hTNF-α more than mTNF-α and hTNF-α more than lymphotoxin (6). Polyclonal antibody to TNF-α Inh immunoprecipitates the TNF receptor complex, and immunofluorescence staining of activated T cells with FITC-coupled TNF-α parallels immunofluorescence staining of the same cells with anti-TNF-α Inh. The complete amino acid sequence of the receptor and inhibitor will establish the true relationship between the two proteins. All these results taken together emphasize the concept that the TNF-α Inh originally described (6) might be a soluble form of the TNF receptor itself.

This work was supported by the Swiss National Science Foundation (Grant 31.-26426-89), the Foundation Carlos and Elsie de Reuter Medical Research Center, and GLAXO IMB, Geneva.

1. Old, L. J. (1985) *Science* **230**, 630–632.
2. Beutler, B. & Cerami, A. (1986) *Nature (London)* **320**, 584–588.
3. Beutler, B. & Cerami, A. (1988) *Annu. Rev. Biochem.* **57**, 505–518.
4. Aggarwal, B. B., Eessalu, T. E. & Hass, P. E. (1985) *Nature (London)* **318**, 665–667.
5. Stauber, G. B., Aiyer, R. A. & Aggarwal, B. B. (1988) *J. Biol. Chem.* **263**, 19098–19104.
6. Seckinger, P., Isaaz, S. & Dayer, J.-M. (1988) *J. Exp. Med.* **167**, 1511–1516.
7. Seckinger, P., Isaaz, S. & Dayer, J.-M. (1989) *J. Biol. Chem.* **264**, 11966–11973.
8. Seckinger, P., Vey, E., Turcatti, G., Wingfield, P. & Dayer, J. M. (1990) *Eur. J. Immunol.*, in press.
9. Engelmann, H., Aderka, D., Rubinstein, M., Rotman, D. & Wallach, D. (1989) *J. Biol. Chem.* **264**, 11974–11980.
10. Olsson, I., Lantz, M., Nilsson, E., Peetre, C., Thysell, H., Grubb, A. & Adolf, G. (1989) *Eur. J. Haematol.* **42**, 270–275.
11. Dayer, J.-M. & Seckinger, P. (1989) in *Interleukin-1 and Diseases*, eds. Bomford, R. H. R. & Henderson, B. (Elsevier, Amsterdam), Chap. 16, pp. 282–302.
12. Mosley, B., Beckmann, M. P., March, C. J., Idzerda, R. L., Gimpel, S. D., VandenBos, T., Friend, D., Alpert, A., Anderson, D., Jackson, J., Wignall, J. M., Smith, C., Gallis, B., Sims, J. E., Urdal, D., Widmer, M. B., Cosman, D. & Park, L. S. (1989) *Cell* **59**, 335–348.
13. Thiele, D. L., Kurosaka, M. & Lipsky, P. E. (1983) *J. Immunol.* **131**, 2282–2290.
14. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
15. Shirakawa, F., Tanaka, Y., Ota, T., Suzuki, H., Eto, S. & Yamashita, U. (1987) *J. Immunol.* **138**, 4243–4248.
16. Owen-Schaub, L. B., Crump, W. L., III, Morin, G. I. & Grimm, E. A. (1989) *J. Immunol.* **143**, 2236–2241.
17. Pennica, D., Hayflick, J. S., Bringman, T. S., Palladino, M. A. & Goeddel, D. V. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6060–6064.
18. Pennica, D., Nedwin, G. E., Hayflick, J. S., Seeburg, P. H., Derynck, R., Palladino, M. A., Kohr, W. J., Aggarwal, B. B. & Goeddel, D. V. (1984) *Nature (London)* **312**, 724–729.
19. Jones, E. Y., Stuart, D. I. & Walker, N. P. C. (1989) *Nature (London)* **338**, 225–228.
20. Stauber, G. B. & Aggarwal, B. B. (1989) *J. Biol. Chem.* **264**, 3573–3576.
21. Ranges, G. R., Zlotnik, A., Espevik, T., Dinarello, C. A., Cerami, A. & Palladino, M. A., Jr. (1988) *J. Exp. Med.* **167**, 1472–1478.
22. Kramer, S. M., Aggarwal, B. B., Eessalu, T. E., McCabe, S. M., Ferraiolo, B. L., Figari, I. S. & Palladino, M. A., Jr. (1988) *Cancer Res.* **48**, 920–925.
23. Wingfield, P., Pain, R. H. & Graig, S. (1987) *FEBS Lett.* **211**, 179–184.
24. Smith, R. A. & Baglioni, C. (1987) *J. Biol. Chem.* **262**, 6951–6954.
25. Lewit-Bentley, A., Fourme, R., Kahn, R., Prangé, E., Waschett, P., Tavernier, J. & Hauquier, G. (1988) *J. Mol. Biol.* **199**, 389–392.
26. Eck, M. J. & Sprang, S. R. (1989) *J. Biol. Chem.* **29**, 17595–17605.
27. Novick, D., Engelmann, H., Wallach, D. & Rubinstein, M. (1989) *J. Exp. Med.* **170**, 1409–1414.
28. Hohmann, H. P., Remy, R., Brockhaus, M. & von Loon, A. P. G. M. (1989) *J. Biol. Chem.* **264**, 14927–14934.
29. Engelmann, H., Novick, D. & Wallach, D. (1990) *J. Biol. Chem.* **265**, 1531–1536.
30. Scheurich, P., Thoma, B., Ücer, U. & Pfizenmaier, K. (1987) *J. Immunol.* **138**, 1786–1790.
31. Israel, S., Hahn, T., Holtmann, H. & Wallach, D. (1986) *Immunol. Lett.* **12**, 217–224.
32. Kull, F. C., Jr., Jacobs, S. & Cuatrecasas, P. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 5756–5760.
33. Scheurich, P., Ücer, U., Krönke, M. & Pfizenmaier, K. (1986) *Int. J. Cancer* **38**, 127–133.