Modulation of Two Forms of Tumor Necrosis Factor Receptors and Their Cellular Response by Soluble Receptors and Their Monoclonal Antibodies*

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Recently, two different receptors for human tumor necrosis factor (TNF) with molecular masses of 60 kDa (p60) and 80 kDa (p80) have been identified. In this report, we investigated the effect of the soluble forms of these receptors and monoclonal antibodies against them on ligand interaction, receptor down-regulation, and mediation of cellular response in U-937 cells. Our results indicate that p60 and p80 constitute 20-30 and 60-80% of the total TNF-binding sites on U-937 cells, respectively. However, by cross-linking, only the p80 form of the receptor could be detected. In contrast to unlabeled TNF, the anti-p60 and anti-p80 antibodies together only partially inhibited ligand binding, and this inhibition was not additive. Lack of additive inhibition of binding was found to be not due to stereoch- emical hindrance. TNF binding to cells can be completely displaced by soluble forms of either the p60 or p80 receptor. However, 100-fold more of the p80 than the p60 form of the soluble receptor is needed for equivalent displacement. Under optimum conditions, TNF and the anti-p80 and anti-p60 antibodies down-regulated 30, 80, and 20% of the TNF receptors, respectively. The anti-p60 and anti-p80 antibodies down-regulated not only their own receptors, but also reciprocal receptors, suggesting a cross-communication between the p60 and p80 forms of the TNF receptor.

In spite of inhibiting as much as 80% of TNF binding, none of the receptor antibodies significantly inhibited the cytotoxic response to TNF in U-937 cells. Soluble forms of both receptors, however, completely abrogated the cellular response to TNF. Thus, overall, our results indicate that the antibodies against both receptors together inhibit the majority of the receptor-ligand interaction without any significant effect on the biological response to TNF.

Tumor necrosis factor (TNF), a protein with a molecular mass of 17 kDa, was originally described as a product of activated macrophages and shown to display tumoricidal activity (Carswell et al., 1975; Aggarwal et al., 1986; Pennica et al., 1984). Extensive research during the last few years has made it apparent that TNF is a highly pleiotropic cytokine and may play a role in septic shock, cachexia, inflammation, autoimmunity, and other immunological and pathological reactions (Tracey et al., 1986; Le and Vilcek, 1987; Aiyer et al., 1987; Goeddel et al., 1989). TNF interacts with a wide variety of cell types by binding to its specific receptors on the cell surface. Upon binding to its receptors, TNF is rapidly internalized and subsequently degraded with slower kinetics (Teijimo et al., 1985). Studies using microinjection of TNF into target cells (Smith et al., 1990) and also inhibition of TNF cytotoxicity by agents that disrupt the cytoskeleton or inhibit lysosomal activity (Kull and Cuatrecasas, 1981) suggest that TNF must be internalized to exert its biological effects. However, there is also a report demonstrating that TNF internalization is not essential for its signal transduction (Perez et al., 1990). The receptor for TNF can be up-regulated by a large number of agents, including interferons, dibutyryl cAMP, retinal, butyrate, TNF, thyroid-stimulating hormone, and lectins (Aggarwal et al., 1985b, 1986; Teijimo et al., 1985; Sheurich et al., 1989; Pang 1989), and down-regulated by interleukin-1, granulocyte/monocyte colony-stimulating factor, phorbol esters, glucocorticoids, microtubule-depolymerizing agents, and lipopolysaccharides (Ruggiero and Baglioni, 1987; Holtmann and Wallach, 1987; Pouteau and Natahn, 1990; Aggarwal and Eessalu, 1987b; Kull, 1988; Ding et al., 1989).

Recently, the cDNAs for two different TNF receptors with approximate molecular masses of 60 and 80 kDa have been isolated (Loetscher et al., 1990; Schall et al., 1990; Smith et al., 1990). Even though these two receptors display a similar architectural structure, <25% overall amino acid sequence identity was noted. Most of the sequence homology is displayed in the extracellular domain, whereas no homology is observed in the cytoplasmic domain (Dembic et al., 1990). The lack of relatedness in the cytoplasmic domain suggests that the two receptors activate different intracellular signaling pathways. The p80 form of the receptor, also referred to as type A, is present primarily in myeloid cells, whereas p60 or type B receptors have been shown to be present in cells of epithelial origin (Hohmann et al., 1989). Furthermore, it has been shown that the synthesis of these two types of the receptor is independently regulated (Hohmann et al., 1990; Eriksen et al., 1991).

Why two distinct receptors for TNF should exist is not clear. Unlike the other two-chain receptors such as IL-2, the cross-linking of two TNF receptors does not appear to be...
required to constitute the high affinity receptor (Hatakeyama et al., 1989); each receptor type appears to bind TNF with high affinity. Transduction of the same signal has been demonstrated by both types of the receptor (Hohmann et al., 1990). To understand the relationship between the two types of the TNF receptor, we investigated these receptors in U-937 cells with respect to ligand interaction and ligand-induced down-regulation and their role in the mediation of the cellular response. Our results show a lack of complete inhibition of TNF by both antibodies together directed against the p60 and p80 forms of the receptor, and down-regulation studies suggest receptor cross-down-regulation. Even though these antibodies blocked most of the TNF binding, they were unable to inhibit the TNF-dependent cytotoxic response.

**EXPERIMENTAL PROCEDURES**

**Materials**—Penicillin, streptomycin, RPMI 1640 medium, and fetal calf serum were obtained from GIBCO. Carrier-free Na125I was purchased from Amersham Corp. IODO-GEN (tetrahydrodiphenylglycouril), glycine, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), NaCl, bovine serum albumin, gelatin, Triton X-100, aprotinin, bacitracin, phenylmethylsulfonyl fluoride, polyethylene glycol 6000, and thimerosal were obtained from Sigma. Bacteria-derived recombinant human TNF, purified to homogeneity with a specific activity of $5 \times 10^6$ units/mg, was kindly provided by Genentech Inc. (South San Francisco, CA). The cross-linking reagent EGS was obtained from Pierce Chemical Co. Molecular weight standards for SDS-PAGE, PD-10 columns, and protein A-Sepharose was obtained from Pharmacia LKB Biotechnology Inc.

**Soluble Receptors and Monoclonal Antibodies**—The recombimant extracellular domains of TNF receptors p60 (TNF binding protein I) and p80 (TNF binding protein II) with molecular masses of 30 and 40 kDa, respectively, were kindly provided by Dr. Tadahiko Kohno (Synergen Inc., Boulder, CO). The soluble forms of these receptors have been characterized (Kohno et al., 1990). Monoclonal antibodies htr-9 and htr-1, specific against the type B/p55/p60 and type A/p75/p80 forms of the TNF receptors, respectively, were kindly provided by Dr. Manfred Brockhaus (F. Hoffmann-La Roche AG, Basel, Switzerland). The characterization of these antibodies has been published (Brockhaus et al., 1990). Both of these antibodies belong to the IgG1 isotype.

**Cell Line**—The human histiocytic cell line U-937 (ATCC CCL-159), human embryonic lung fibroblast cell line WI-38/VAF transformed with SV40 (ATCC CCL-185), human embryonic liver carcinoma cell A-549 (ATCC CCL-185), human breast adenocarcinoma cell line MCF-7 (ATCC HTB-22) were grown in RPMI 1640 medium supplemented with fetal calf serum (10%), penicillin (100 U/ml), and streptomycin (100 µg/ml). Briefly, the cells were seeded at a density of $1 \times 10^5$/ml in T-25 flasks (Falcon 3001), Becton Dickinson Labware, Lincoln Park, NJ) containing 10 ml of medium and grown at 37 °C in an atmosphere of 95% air and 5% CO2. Cell cultures were split every 3 days.

**Iodination of TNF and Anti-p60 and Anti-p80 Antibodies**—Recombinant human TNF and the anti-p60 and anti-p80 antibodies were labeled with Na125I using the IODO-GEN procedure as described previously (Aggarwal et al., 1985). This method specifically labels a single tyrosine residue in TNF at position 87 (Aggarwal and Eesalu, 1989). The cross-linking reagent EGS freshly prepared in dimethyl sulfoxide. After incubation for 40 min at 4 °C with gentle agitation, the unreacted cross-linking reagent was quenched with 20 mM ammonium chloride (final concentration). After another 10 min, the cells were washed with cold PBS and then solubilized in solubilization buffer (50 mM Tris (pH 7.5) supplemented with aprotinin (0.2 mg/ml), phenylmethylsulfonyl fluoride (1 mM), bacitracin (0.1%), and Triton X-100 (1% (v/v) final concentration)).

**Specific Immunoprecipitation of Cross-linked TNF Receptor**—The receptor, covalently cross-linked to the ligand, was immunoprecipitated using a combination of rabbit anti-TNF-α IgG and protein A-Sepharose. First, the clarified cell extract was precleared at 4 °C for 90 min with protein A-Sepharose equilibrated with PBS containing 0.1% Triton X-100 (equilibration buffer) to remove proteins that might bind nonspecifically to the resin. The unbound fraction was then incubated with anti-TNF-α antibody for 12 h at 4 °C, followed by binding to a fresh aliquot of protein A-Sepharose for 90 min at 4 °C. The immune complexes were collected by centrifugation at 1000 × g for 5 min and washed five times with 20 volumes of equilibration buffer. The receptor-ligand complexes were resuspended in SDS sample buffer and then analyzed by SDS-PAGE.

**Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis**—Samples containing 125I-TNF-α-labeled receptors were electrophoresed in the presence of 0.1% SDS on 5–15% acrylamide gradient gels using the method of Laemmli (1970) at a constant current of 25 mA. Unless otherwise stated, all samples to be electrophoresed were first reduced with 50 mM dithiothreitol for 2 min at 60 °C. Prestained molecular weight standards were used to identify the approximate region of the gel where labeled receptor would be located. After electrophoresis, the gel was immediately fixed in a solution of 40% methanol and 10% acetic acid. Protein was stained by staining with silver or Coomassie Brilliant Blue. The gels were dried between cellophane membranes before autoradiography. Radioactively labeled proteins were visualized by exposing the dried gel to Kodak x-ray film with Cronex intensifying screens at −70 °C.

**Cytotoxicity Assay**—The cytotoxicity assay was done using the modified tetrazolium salt (MTT) assay described (Hansen et al., 1989). Briefly, U-937 cells (5000/well) were incubated in the presence or absence of the indicated test sample in a final volume of 0.2 ml for 72 h at 37 °C. Thereafter, 0.1 ml of cell medium was removed, and 0.025 ml of MTT solution (5 mg/ml in PBS) was added to each well. After a 4 h incubation at 37 °C, 0.1 ml of extraction buffer (20% sodium dodecyl sulfate, 50% dimethylformamide) was added. After overnight incubation at 37 °C, the absorbances at 570 nm were measured using a 96-well multispecimen autoreader (Dynatech MR 5000) with extraction buffer as a blank: % cytotoxicity = ($A_{570}$ of treated sample/$A_{570}$ of untreated sample)) × 100.

**RESULTS**

**Characterization of Binding of Anti-p60 and Anti-p80 Antibodies with Two Types of TNF Receptor in U-937 Cells**—Anti-p60 and anti-p80 are monoclonal antibodies raised against each type of the TNF receptor, and they both belong to the IgG1 isotype. We investigated the ability of these antibodies to block the binding of labeled TNF to U-937 cells. As shown in Fig. 1, anti-p60 blocked >30% of the total TNF binding, and anti-p80 inhibited TNF binding by ~70%. As a control, unlabeled TNF inhibited ~85% of total binding.
Unrelated monoclonal antibody (against interferon-γ) of the IgG1 isotype had no effect on TNF binding. The dose-response curves shown in Fig. 2 indicate that additional amounts of antibodies did not enhance the inhibition of TNF binding. Thus, these results suggest that 20–30% of the total receptors present in U-937 cells are in the p60 form, whereas as much as 60–80% of the binding of TNF to the cells is due to the p80 form of the receptor.

Since unlabeled TNF almost completely abolished the binding of labeled TNF to U-937 cells, we investigated the ability of anti-p60 and anti-p80 together to completely inhibit TNF binding. The results shown in Fig. 3 indicate that inhibition of TNF binding by the two antibodies together was not additive even at saturating concentrations of the antibody (5 μg/ml for each; see Fig. 2). Furthermore, even both antibodies together could not completely inhibit TNF binding. These results suggest binding sites that cannot be neutralized by antibodies to either type of the receptor on the surface of U-937 cells. The inability of anti-p60 and anti-p80 to completely inhibit both the receptors could also be due to stereochemical hindrance. Experiments were carried out to investigate this possibility. Both anti-p60 and anti-p80 antibodies were radiiodinated, and their direct binding to the cells was examined. The results of these experiments are shown in Fig. 4. As demonstrated (left panel), the binding of labeled anti-p60 could be inhibited only by unlabeled anti-p60, but not by anti-p80, suggesting that anti-p80 does not interfere with the binding of anti-p60. Similarly, the reciprocal experiment shown (right panel) suggests that labeled anti-p80 binding could be blocked only by anti-p80, and not by anti-p60. These results rule out the possibility that stereochemical hindrance causes the inability of anti-p60 and anti-p80 together to completely block TNF binding. The lack of complete inhibition could also be due to lack of specificity of antibodies for their own antigen. Therefore, experiments were carried out to determine the antibody specificity for each receptor by its ability to be displaced by the soluble form of each receptor.

The results of these experiments are shown in Fig. 5. As shown (left panel), labeled anti-p60 bound to U-937 cells, and its binding could be displaced only by the soluble form of the p60 antigen, and not by the p80 antigen. Similarly, as shown (right panel), anti-p80 binding was specific for the p80 form of the antigen. Thus, the inability of both antibodies together to inhibit TNF binding was not due to cross-reactivity of the antibodies.

The interaction of the p60 and p80 forms of the receptor with the ligand was further examined by the ability of a soluble form of each receptor to inhibit TNF binding to U-937 cells. The results of these experiments are shown in Fig. 6. It is clear that soluble p60 and p80 independently could completely displace the binding of labeled TNF similar to unlabeled ligand. It appears that at lower concentrations, p80 enhanced TNF binding (up to 30%) to the cells; however, this
increase was found to be not reproducible in subsequent experiments. Furthermore, since 100 times more soluble p80 than p60 or TNF was needed to completely inhibit ligand binding to U-937 cells, the p80 form of the receptor may have a 100-fold lower affinity than the p60 receptor for TNF.

Down-regulation of p60 and p80 Forms of TNF Receptor

We also investigated the ability of anti-p60 and anti-p80 to down-regulate their own receptors. The results of these experiments are shown in Fig. 7. Anti-p60 could down-regulate maximally up to 25% of the total TNF receptors, whereas anti-p80 induced almost 80% down-regulation of the receptor. Interestingly, TNF induced only a partial (up to 50%) down-regulation of its receptor. But the dose responses of down-regulation by TNF and anti-p60 were quite different. The type of the receptor down-regulated by TNF, anti-p60, and anti-p80 was also investigated. The results of these experiments are shown in Table I. The results clearly indicate that TNF completely down-regulated the p60 form of the TNF receptor and only partially down-regulated the p80 form (36%). Besides completely down-regulating its own receptor, anti-p80 also significantly (72%) down-regulated the p60 receptor. Similarly, besides its own receptor, anti-p60 down-regulated the p80 receptor. This kind of cross-down-regulation suggests a communication between the two kinds of the TNF receptor in U-937 cells.

Receptor-Ligand Cross-linking Studies

To further determine association/interaction between the two types of the TNF receptor, we carried out receptor-ligand cross-linking studies with several different cell types. The results of these experiments are shown in Fig. 8. In U-937 cells, a single band at an approximate molecular mass of 98 kDa was observed that disappeared on addition of an excess of unlabeled TNF. If one assumes that it is the monomeric form of TNF that interacts with the receptor, then the molecular mass of the receptor cross-linked to the ligand is 80 kDa. Thus, no band was observed due to the cross-linking of the ligand to the 60-kDa form of the receptor. However, in the transformed human fibroblast cell line WI-26VAF, three bands at approximate molecular masses of 98, 78, and 70 kDa were observed. The 98 and 78-kDa bands correspond to the p90 and p60 forms of the receptor, whereas the 70-kDa form may represent either a novel receptor or the breakdown product of the previous two receptors. Three bands after cross-linking were also observed in A-549 cells, a human lung carcinoma cell line. In the human breast tumor cell line MCF-7, a single major band at 78 kDa was observed that corresponds to a molecular mass of ~60 kDa. From these cross-linking studies, no evidence of a physical association between the p60 and p80 forms of the receptor was found either in the absence or presence of the ligand. We also carried out gel filtration studies to determine the physical association between the two types of the receptor both in the presence and absence of the ligand. However, no evidence for association was found (data not shown).

Effect of Anti-p60 and Anti-p80 Receptor Antibodies on Cellular Response to TNF

To understand the effect of these antibodies on the cellular response to TNF, we investigated the cytotoxic response to TNF in comparison to anti-p60 and anti-p80 in U-937 cells. The results of these experiments are shown in Fig. 9. TNF inhibited the proliferation of these cells in a dose-dependent manner. Anti-p60 was found to be a partial agonist of TNF, whereas anti-p80 was minimally active. A suboptimal response observed with anti-p60 was found to be not due to the induction of TNF in U-937 cells by this antibody (data not shown). Since both antibodies inhibited TNF binding to a variable extent, their ability to affect the cytotoxic response to TNF was investigated. The results
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Fig. 5. Dose-dependent inhibition of labeled anti-p60 or anti-p80 antibody binding in U-937 cells. Cells (1 x 10^6) were incubated with either the ^125I-labeled anti-p60 antibody (0.8 x 10^6 cpm) (A) or the ^125I-labeled anti-p80 antibody (0.2 x 10^6 cpm) (B) in the presence of the indicated concentrations of p60 or p80 at 4 °C for 1 h. Thereafter, binding was assayed as described under "Experimental Procedures." All determinations were made in triplicate. 100% TNF binding is 338 ± 41 and 798 ± 14 cpm (A and B, respectively).

shown in Table II indicate that even though anti-p80 blocked as much as 80% of TNF binding, it did not inhibit the cellular response to TNF. Instead, a small but significant enhancement (61.8 versus 70.4%) of the response to TNF by anti-p80 was observed. Similarly, even though anti-p60 also inhibited ligand binding and was a partial agonist, it had no significant effect on the cytotoxic response to TNF. The lack of effect of these antibodies on the biological response to TNF suggests that perhaps very few receptors are needed for the mediation of the cellular response and that these functional receptors are not recognized by either of the antibodies. Since the soluble forms of the p60 and p80 receptors completely inhibited ligand binding (Fig. 6), we also investigated their ability to inhibit the biological effects of TNF. As shown in Table II, the results clearly indicate that the soluble receptors independently can inhibit the cytotoxic response to TNF, thus suggesting their usefulness as inhibitors of the harmful effects of TNF.

**DISCUSSION**

Recently, two different TNF receptors with homologous extracellular domains (25%) and heterologous cytoplasmic domains have been identified (Dembic et al., 1990; Smith et al., 1990; Loetscher et al., 1990; Schall et al., 1990). Since each receptor binds the ligand with high affinity and transduces the signal (Hohmann et al., 1990), it is not clear why there should be two distinct chains of the TNF receptor. The lack of structural identity in the intracellular domain between the two receptors suggests a difference in signal transduction. In the murine system, the p60 form of the receptor has been shown to be responsible for signaling cytotoxicity and induction of various genes, whereas the p80 form of the receptor signals proliferation in primary thymocytes and in a cytotoxic T cell line (Tartaglia et al., 1991). However, there has been no direct evidence in the human system to support this suggestion. In this report, we investigated the effect of monoclonal antibodies against these two receptors on the interaction of TNF and the mediation of the cellular response. Our results indicate that U-937 cells express both the p60 and p80 forms of the TNF receptor. Based on monoclonal antibodies specific to each type of the receptor, we found that ~30% of total TNF binding to U-937 cells is due to p60 and that 70% is due to p80. These results are consistent with a previous report (Espevik et al., 1990). We also found that TNF binding could not be completely inhibited by the antibodies together and that inhibition was not additive. These results were not due to stereochemical hindrance because direct binding of one antibody to the receptor on the cell surface was not blocked by the other. The results also did not appear to be due to the lack of specificity of antibodies. These results raise the possibility of a third type of the TNF receptor. We also found evidence of receptor cross-down-regulation of the two types of the TNF receptor. It appears that anti-p80 not only down-regulates p80, but also p60. Similarly, anti-p60 down-regulated both receptors. The cross-down-modulation may occur through transduction of a signal by one receptor to the other or there may be a physical association between the two receptors. Both cross-linking and gel filtration studies showed no evidence of physical association. The cross-linking experiments on cells whose surface proteins are labeled either by...
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**FIG. 6.** Displacement of labeled TNF binding by TNF and soluble p60 and p80 forms of receptor in U-937 cells. Cells (5 x 10⁵/well) were incubated with ¹²⁵I-labeled TNF in the presence of the indicated concentrations of p60, p80, or TNF at 4°C for 1 h, and then cell-bound radioactive ligand was assayed as described under "Experimental Procedures." All determinations were made in triplicate. 100% TNF binding is 2319 ± 136 cpm.

**FIG. 7.** Down-regulation of TNF receptors by TNF and anti-p60 and anti-p80 antibodies in U-937 cells. Cells (5 x 10⁵/well) were incubated with the indicated concentrations of TNF or the anti-p60 or anti-p80 antibody at 37°C for 1 h. Then the medium was removed, and cells were washed twice with binding buffer. Cell-bound TNF or antibodies were removed by acid treatment as described under "Experimental Procedures." Then the remaining receptors on the cell surface were assayed as described. 100% TNF binding is 5466 ± 139 cpm.

radioiodination or by [³⁵S]methionine incorporation also failed to reveal any direct physical association between the two types of the TNF receptor either in the presence or absence of the ligand. Therefore, it is possible that cross-down-regulation of the receptors may occur indirectly through transduction of a signal from one receptor to the other.

By cross-linking, we could detect only the p80 form, and not the p60 form, of the TNF receptor in U-937 cells. These results are consistent with a previously published report (Staubé et al., 1988). The reason for cross-linking of the ligand preferentially to only one form of the receptor while both are present is not clear. It is not due to the receptor affinity since both forms have very similar affinities for the ligand. However, it could be due to the receptor density since there is 2–3 times as much p80 receptor as p60 receptor. In MCF-7 cells, we could detect only the p60 form, and not the p80 form, of the receptor, whereas both forms of the receptor could be detected in A-549 and WI-26/VAF cells. These results are in agreement with those of Hohmann et al. (1989).

Our results differ from those of Brockhaus et al. (1990), who showed complete inhibition of ligand binding in the presence of both antibodies. The difference between the two results may be due to experimental conditions. The previous workers carried out preincubation of cells with antibodies at 37°C, washed the cells, and then examined ligand binding at 4°C. These conditions can mediate not only receptor blocking, but also receptor down-regulation.

Our results also suggest that the ligand-binding regions in the extracellular domains of the p60 and p80 receptors are very similar since the binding of TNF to cells could be completely displaced by the soluble form of either the p60 or

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**TABLE I**

Cross-down-regulation of the p60 and p80 forms of the TNF receptor in U-937 cells by TNF and the anti-p60 and anti-p80 antibodies

| Treatment         | Down-regulation p60 | Down-regulation p80 |
|-------------------|----------------------|---------------------|
| TNF               | 100                  | 35.8                |
| Anti-p60          | 68.9                 | 28.9                |
| Anti-p80          | 72.2                 | 99.2                |

**FIG. 8.** SDS-PAGE analysis of receptor-ligand complex in various cell lines after treatment with bifunctional cross-linking reagent. In each case, 10⁷ cells bound to ¹²⁵I-TNF-α were treated at 4°C with freshly prepared EGS (0.1 mM final concentration) for 40 min. Afterwards, the reaction was quenched, and the cells were solubilized as described under "Experimental Procedures." Samples were resolved by SDS-PAGE (5–15% acrylamide gradient) under reducing conditions using a final concentration of 50 mM dithiothreitol. The receptor-ligand complex was then visualized by autoradiography.
p80 receptor (Fig. 6). There are four cysteine-rich domains in the extracellular domain of each receptor, but the amino acid sequence in this region is only 20% identical. It appears that between the two chains of the IL-2 receptor. In contrast, each must be some differences in the ligand-binding site. Even though, as shown here, the soluble form of the p60 soluble form of the p80 receptor. It is possible that the difference in the affinity between soluble p60 and p80 is because the latter is somewhat denatured; however, this is unlikely. Whether antibodies against the p55 and p75 forms of the IL-2 receptor inhibit IL-2 binding in an additive manner and also cross-down-regulate each other as shown here is not known. A two-chain receptor has also been identified for nerve growth factor (Klein et al., 1991). It consists of a polypeptide chain whose extracellular domain is 39% identical to that of the TNF receptor, binds nerve growth factor with low affinity, and does not appear to transduce the signal (Johnson et al., 1986). The second polypeptide chain, which is quite dissimilar, is coded by the trk proto- oncogene, binds nerve growth factor with high affinity, and appears to be responsible for signal transduction independently of the second chain. Thus, the relationship between these low- and high-affinity receptors of nerve grow factor is not yet clear.

We also found that besides inhibiting TNF binding, the anti-p60 antibody had cytotoxic effects on U-937 cells; however, this response was suboptimal compared to TNF. These results are consistent with previous reports (Espevik et al., 1990; Engelmann et al., 1990), suggesting that TNF is not needed for intracellular signaling. The reason for the ability of anti-p60 to only partially activate the receptor for the mediation of the cellular response is not clear. It is possible that the participation of p80 along with p60 is essential for induction of the optimum response. The anti-p80 antibody, in contrast, was found to be not significantly active in this assay. We also found that in contrast to p60, p80 was completely inactive in the mediation of the cytotoxic response in U-937 cells. This confirms the observation of Tartagalia et al. (1991) in the murine system. We found, however, that for full activation of the receptor, TNF was needed. Interestingly, the anti-p80 antibody, which inhibited receptor binding as much as 80% of TNF binding, had no effect on the biological response of cells to TNF. These results also suggest that the p80 form of the receptor is perhaps not directly involved in signal transduction. Anti-p60, which inhibits 20–30% of total TNF binding, also did not block the cytotoxic response to TNF. This may be because this antibody by itself is a partial agonist of TNF.

Our results differ from previous reports indicating that both the p60 and p80 forms of the TNF receptor can transduce the signal (Hohmann et al., 1990). The difference may be in the assay systems and cell types used. Hohmann et al. (1990) employed Hep2 cells to study p60 and HL-60 cells to study p80, and the induction of transcription factor NF-κB was used to determine the biological response. These workers used anti-p60 as a ligand to investigate the activity of the p60 receptor directly, whereas the activity of the p80 form of the receptor was examined indirectly by the ability of the anti-p80 antibody to inhibit the TNF-induced biological response. The evidence that the p60 form, but not the p80 form, of the TNF receptor alone can transduce the signal in certain systems is consistent with another report based on the effects of murine and human TNFs on murine cell lines (Lewis et al., 1991). The p80 form of the TNF receptor was shown to be species-specific, whereas the p60 form was reported to mediate the response of both murine and human TNFs. Using receptor-blocking antibodies, it was concluded by Thomas et al. (1990) that ligand binding to the p80 form of the TNF receptor is not sufficient for signal transduction. Even though the p80 form of the TNF receptor may not be directly involved in signal transduction in some systems, it appears to control the activity of p60 as indicated by our studies on complete inhibition of ligand binding and the biological response by the soluble form of the p80 receptor and also by studies on receptor cross-down-regulation.

Consistent with binding studies, we found that the soluble forms of both receptors could independently block the biological response to TNF. Thus, even though the p80 form of the TNF receptor may differ from p60 with respect to its ability to transduce the signal, it could serve as a full competitive antagonist, although it is less efficient than p60. Since it has been shown that the synthesis of the two receptors is regulated...
independently, the difference in the biological response may be determined by the levels of the p60 form of the TNF receptor. To further understand the role of the individual TNF receptors and their interaction, we have begun studies with receptor-negative cells transfected with the recombinant form of the molecule.

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