Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 9292–9296, October 1991
Immunology

The two different receptors for tumor necrosis factor mediate distinct cellular responses

(species specificity/antibodies/manganous superoxide dismutase/cytotoxicity/proliferation)

LOUIS A. TARTAGLIA*, RICHARD F. WEBER*, IRENE S. FIGARI†, CARMEN REYNOLDS†, MICHAEL A. PALLADINO, JR.†, AND DAVID V. GOEDDEL*

Departments of *Molecular Biology and †Cell Biology, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, CA 94080

Communicated by Bruce N. Ames, July 25, 1991

ABSTRACT **The individual roles of the murine type 1 and type 2 tumor necrosis factor (TNF) receptors (TNF-R1 and TNF-R2) were investigated utilizing (*i*) the strong species specificity of TNF-R2 for murine TNF compared to human TNF and (*ii*) agonistic rabbit polyclonal antibodies directed against the individual TNF receptors. Proliferation of mouse thymocytes and the murine cytotoxic T-cell line CT-6 is stimulated by murine TNF but not by human TNF. Consistent with this observation, polyclonal antibodies directed against TNF-R2 induced proliferation in both of these cell types, whereas polyclonal antibodies directed against TNF-R1 had no effect. In contrast, cytotoxicity in murine LM cells (which are sensitive to murine and human TNF) was induced by antibodies against TNF-R1 but not by antibodies against TNF-R2. Also, the steady-state level of manganous superoxide dismutase mRNA in the murine NIH 3T3 cell line was induced by murine TNF, human TNF, and anti-TNF-R1 but not by anti-TNF-R2. These results suggest that TNF-R2 initiates signals for the proliferation of thymocytes and cytotoxic T cells, whereas TNF-R1 initiates signals for cytotoxicity and the induction of the protective activity, manganous superoxide dismutase. The nonredundant signaling observed for the two TNF receptors cannot be explained simply by the differential expression of the two TNF receptors in the various cell types, because LM cells express on their surface higher levels of TNF-R2 than TNF-R1, and LM cells, NIH 3T3 cells, and thymus cells all express mRNA corresponding to both receptor types. It is therefore likely that the two receptors initiate distinct signaling pathways that result in the induction of different cellular responses.**

Tumor necrosis factor (TNF) is a multifunctional cytokine produced mainly by activated macrophages, T cells, mast cells, and some epithelial tumor cells (1–3). The wide range of biological effects elicited by TNF include hemorrhagic necrosis of transplanted tumors, growth proliferation of normal cells, cytotoxicity, inflammatory, immunoregulatory and antiviral responses, and an important role in endotoxic shock (4–8). The first step in the induction of these various cellular responses by TNF is the binding to specific cell surface receptors. TNF receptors have been detected on a wide variety of normal tissues and cell lines that are sensitive or resistant to TNF (9–12). Two immunologically distinct TNF receptors of approximately 55 kDa (TNF-R1) and 75 kDa (TNF-R2) have now been identified (13–16), and human and mouse cDNAs corresponding to both receptor types have been isolated and characterized (17–20).

A number of recent reports have described initial studies investigating the individual roles of the two human TNF receptors. Polyclonal and monoclonal antibodies directed against human TNF-R1 have been shown to behave as receptor agonists and elicit several TNF activities, such as cytotoxicity, fibroblast proliferation, resistance to chlamidiae, and synthesis of prostaglandin E_2 (15, 21, 22). Monoclonal antibodies against human TNF-R1 that block the binding of TNF to TNF-R1 and antagonize several TNF effects have also been described (21–23). Although no direct signaling role for TNF-R2 has yet been identified with either receptor agonists or transfection studies, several reports have described monoclonal antibodies directed against TNF-R2 that can partially antagonize TNF responses (such as cytotoxicity and activation of NF-κB) and enhance the antagonistic effects of anti-TNF-R1 monoclonal antibodies (22–24). These reports suggested that both TNF receptors are active in signal transduction and that there is redundancy in the function of the two receptors. However, the reported effects of the TNF-R2 antagonists have been quite small and were observed exclusively at very low TNF concentrations. It is therefore possible that TNF-R2 is only participating as a minor accessory component to TNF-R1 in the signaling of these responses. In this report we describe a direct role of TNF-R2 in stimulating proliferation of murine thymocytes and T cells and show that this receptor is distinct from the receptor (TNF-R1) mediating cytotoxicity.

MATERIALS AND METHODS

Reagents. Recombinant murine TNF (muTNF) and recombinant human TNF (hTNF) (specific activity $>10^7$ units/mg) were provided by the Genentech manufacturing group. Rabbit anti-murine TNF-R1 and rabbit anti-murine TNF-R2 antibodies were generated against the soluble extracellular domain of the corresponding receptors (ref. 20; R.F.W. and D.V.G., unpublished results). The titers of these antisera were quantitated by a direct antigen-coated ELISA. The dilutions of anti-TNF-R1 and anti-TNF-R2 giving 50% binding to the corresponding purified soluble receptor were 1:109,000 and 1:104,000, respectively. The cross-reactive titers of TNF-R1 antiserum to soluble TNF-R2 and TNF-R2 antiserum to soluble TNF-R1 were <1:200. C3H/HeJ mice (The Jackson Laboratory) were used as the source of fresh thymocytes.

Thymocyte Proliferation Assay. C3H/HeJ thymocytes were cultured in 96-well flat-bottomed culture plates (1.5×10^6 per 0.1 ml) (Costar) in Eagle's minimal essential medium supplemented with 10% heat-inactivated fetal bovine serum (HyClone), 1% L-glutamine, 1% nonessential amino acids, 100 units of penicillin per ml, 100 μg of streptomycin per ml, 0.1% gentamicin (GIBCO), and 0.05 mM 2-mercaptoethanol (Sigma) in the presence of 0.1% phytohemagglutinin P

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.

Abbreviations: TNF, tumor necrosis factor; muTNF, recombinant murine TNF; hTNF, recombinant human TNF; TNF-R1, TNF receptor type 1; TNF-R2, TNF receptor type 2; MnSOD, manganous superoxide dismutase; PHA-P, phytohemagglutinin P; PAI-1, plasminogen activator inhibitor 1; IL-6, interleukin 6.

(PHA-P; Difco). PHA-P, muTNF, and antibodies were added to a final volume of 0.2 ml. After 60 h at 37°C, cultures were pulsed with 1 μCi of [^{3}H]thymidine (5 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear) for 12 h and harvested onto glass fiber filters (PhD; Cambridge Technology, Watertown, MA); mean [^{3}H]thymidine incorporation (cpm) of triplicate cultures was determined using a liquid scintillation counter (Beckman).

CT-6 Proliferation Assay. CT-6 cells (25) were cultured in 96-well flat-bottomed culture plates (5.0×10^4 per 0.1 ml) (Costar) in RPMI medium supplemented with 10% fetal calf serum (HyClone), 1% L-glutamine, 100 units of penicillin per ml, and 100 μg of streptomycin per ml (GIBCO). muTNF and antisera were added to a final volume of 0.2 ml. After 24 h at 37°C, cultures were pulsed with 1 μCi of [^{3}H]thymidine (5 Ci/mmol) for 4 h and harvested onto glass fiber filters, and mean [^{3}H]thymidine incorporation (cpm) of triplicate cultures was determined.

LM Cytotoxicity Assay. LM cells (2×10^4 cells per well) were seeded into 96-well microtiter plates in 200 μl of medium [RPMI medium supplemented with 10% fetal calf serum, 1% L-glutamine, 100 units of penicillin per ml, 100 μg of streptomycin per ml (GIBCO), and 5 μg of insulin per ml] and incubated 24 h at 37°C in a 5% CO_2 atmosphere. Medium was then brought to 10 μg of cycloheximide per ml and the anti-TNF-R antibodies were added to the wells and serially diluted. The plates were incubated for an additional 16 h and the viable cells were stained with 20% methanol containing 0.5% crystal violet. The dye was eluted with 0.1 M sodium citrate and 50% ethanol and absorbance was measured at 540 nm.

Northern Analysis. Total cytoplasmic RNA was extracted from cells as described (26), electrophoresed on a 1.2% formaldehyde/agarose gel (15 μg of RNA per lane), and transferred to nitrocellulose filters. Filters were hybridized and washed as described (26). The probes used for filter hybridizations were either a random-primed ^{32}P-labeled 0.8-kilobase (kb) fragment (entire coding region) of the manganous superoxide dismutase (MnSOD) cDNA (27) or ^{32}P-labeled oligonucleotides corresponding to the murine genes encoding interleukin 6 (IL-6), plasminogen activator inhibitor 1 (PAI-1), β_2-microglobulin, and c-*fos*. Autoradiography was done at −70°C using Kodak intensifying screens.

RESULTS

Thymocyte and T-Cell Proliferation Is Stimulated by Antibodies to TNF-R2. In a previous report we described the cloning of cDNAs encoding the two murine TNF receptors and showed that TNF-R2 binds muTNF with high affinity but fails to recognize hTNF (20). This observation suggested that the various activities of hTNF reported in mice or in murine cell lines would be mediated by TNF-R1, whereas those activities that displayed a strong species specificity for muTNF might be regulated by TNF-R2. Literature searches revealed that the majority of TNF effects reported in mice or murine cell lines exhibited little species preference. An exception to this was TNF-induced proliferation of murine thymocytes and the cytotoxic T-cell line CT6 (25, 28–30). muTNF concentrations as low as 100 units/ml (<10 ng/ml) could induce a strong proliferative response in both of these cell types, whereas hTNF had no effect even at concentrations of >10,000 units/ml (≈1 μg/ml). To examine more directly the receptor that mediates proliferation in these cell types, we tested highly specific polyclonal antibodies directed against the individual receptors for possible agonist activities.

As shown in Fig. 1*A*, muTNF stimulates C3H/HeJ thymocyte proliferation in the presence of a submitogenic dose of PHA-P. No stimulation is seen with hTNF even at doses as high as 10^5 units/ml (refs. 28 and 29; data not shown). Thymocyte proliferation was also examined in response to various dilutions of polyclonal antibodies directed against murine TNF-R1 and TNF-R2. Antibodies to TNF-R2 strongly stimulated the proliferation of the C3H/HeJ thymocytes even at a dilution factor of 10^4. Polyclonal antibodies directed against TNF-R1 had no effect at any concentration tested despite the similar titer of the two types of antisera (Fig. 1*B*).

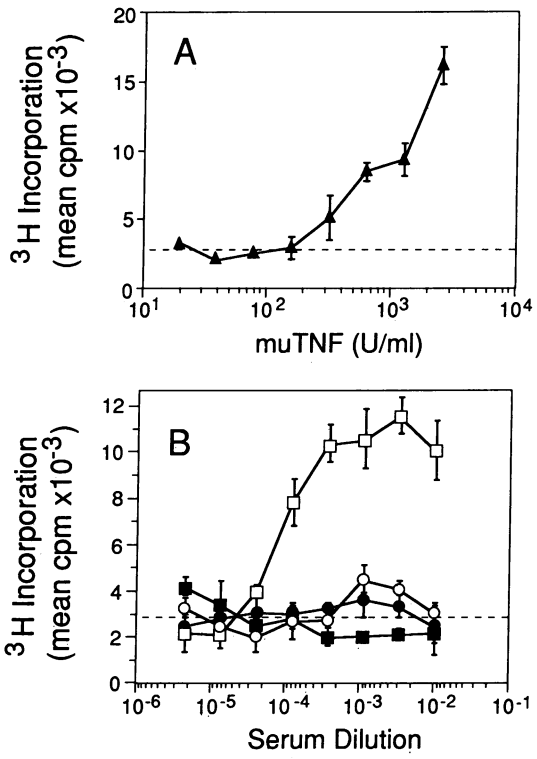

FIG. 1. Proliferation of murine thymocytes in response to muTNF (*A*) and antibodies directed against the murine TNF receptors (*B*). ▲, muTNF; ■, anti-TNF-R1; □, anti-TNF-R2; ●, prebleed-TNF-R1; ○, prebleed-TNF-R2. Thymocytes were cultured for 60 h with a submitogenic dose of PHA-P and the indicated concentrations of muTNF or anti-TNF-R antibody. Cultures were then pulse labeled with [^{3}H]thymidine for 12 h and mean [^{3}H]thymidine incorporation (±SEM) of triplicate cultures was determined. The amount of ^{3}H incorporation in thymocytes treated with PHA-P alone is indicated by a dashed line. No proliferation was observed in the absence of PHA-P.

A similar set of experiments was performed with the interleukin 2 (IL-2)-dependent cytotoxic T-cell line CT6. When IL-2 is removed from the growth medium, muTNF can serve as a proliferative signal (Fig. 2*A*), whereas hTNF cannot (refs. 25 and 30; data not shown). Polyclonal antibodies against TNF-R2 also stimulated the growth of CT6 cells and the magnitude of the proliferative response was similar to that seen with muTNF (Fig. 2*B*). No significant effect was observed with polyclonal antibodies directed against TNF-R1. The agonist activity of the anti-TNF-R2 antibodies in the thymocyte and T-cell proliferation assays indicates that TNF-R2 can signal proliferation in at least some T-cell populations and also that TNF is not required for the signal transmission.

Cytotoxicity in LM Cells Is Induced by Antibodies to TNF-R1. One trivial explanation for the inability of the TNF-R1 antibodies to induce T-cell and thymocyte proliferation was that these antibodies do not possess agonist activity even for those effects that are normally signaled by TNF-R1. It has been shown previously that polyclonal as well as some monoclonal antibodies directed against human TNF-R1 can

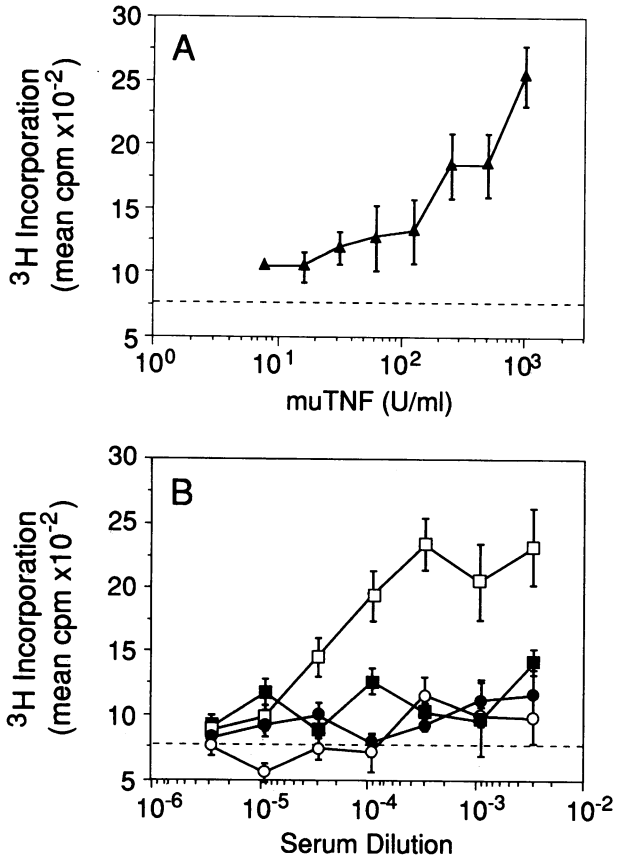

FIG. 2. Proliferation of CT6 cells in response to muTNF (*A*) and antibodies directed against the murine TNF receptors (*B*). ▲, muTNF; ■, anti-TNF-R1; □, anti-TNF-R2; ●, prebleed-TNF-R1; ○, prebleed-TNF-R2. CT6 cells were cultured for 24 h with the indicated concentrations of muTNF or anti-TNF-R antibody. Cultures were then pulse labeled with [^{3}H]thymidine for 4 h and mean [^{3}H]thymidine incorporation (±SEM) of triplicate cultures was determined. The amount of ^{3}H incorporation in untreated CT6 cells is indicated by a dashed line.

induce cytotoxicity in human cell lines (15, 21, 22), although no effect of the human TNF-R1 antibodies has been reported in murine cell lines. To determine if the anti-murine TNF-R1 antibodies could mimic TNF activity, we assayed them for cytotoxicity against murine LM cells. LM cells were chosen because they possess TNF-R1 (40%) and TNF-R2 (60%) (20) and show a similar sensitivity to muTNF and hTNF (indicative of a TNF-R1 response) (30). As shown in Fig. 3, LM cells were highly sensitive to antibodies against TNF-R1 but not TNF-R2. These results indicate that the antibodies to muTNF-R1 are agonistic and that TNF-R1 can mediate cytotoxicity in murine cells. The resistance of LM cells to the TNF-R2 antibodies, despite the cell surface expression of TNF-R2, suggests that TNF-R2 cannot deliver a cytotoxic signal in murine LM cells. Similar TNF-R1 specific cytotoxicity was also seen with the murine cell lines B6MS5, L929, and NIH 3T3 (data not shown).

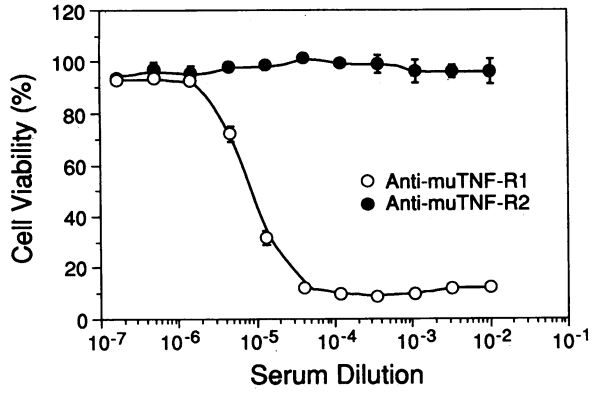

FIG. 3. Cytocidal effect of anti-murine TNF receptor antibodies on LM cells. ○, Anti-TNF-R1; ●, anti-TNF-R2. The antisera were applied for 16 h at the indicated dilutions in the presence of cycloheximide at 10 μg/ml. Viability of cells was determined (see text). Preimmune sera and anti-NGF antiserum had no effect in the range of serum concentrations used in this study. The data shown are the mean of three experiments (±SEM). Error bars have been omitted for points with SEM ≤ the size of symbol (±2%).

TNF Induction of MnSOD mRNA Is Mediated by TNF-R1. The mitochondrial enzyme MnSOD is an important determinant of cellular resistance to the cytotoxic effects of TNF (27). In addition, MnSOD synthesis is specifically and rapidly induced by TNF treatment of many cell types (26). We therefore tested whether induction of the MnSOD gene was mediated by the same or different TNF receptor as the one that signals cytotoxicity. To make a first approximation of the individual roles of TNF-R1 and TNF-R2 in mediating MnSOD induction, we examined the TNF species specificity of this response. As shown in Fig. 4, muTNF and hTNF strongly induced the 1-kb and 4-kb MnSOD transcripts in the murine NIH 3T3 cell line. Both cytokines also induced the steady-state mRNA levels of the genes encoding IL-6, PAI-1, β_2-microglobulin, and c-*fos*. MnSOD mRNA levels were induced by muTNF and hTNF during a short exposure (3 h) in the presence of the protein synthesis inhibitor cycloheximide and a longer exposure (12 h) in the absence of cyclo-

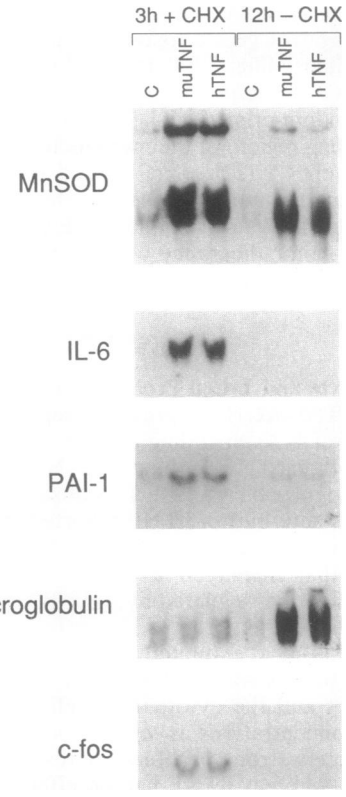

FIG. 4. Induction of mRNA in murine NIH 3T3 cells. The left three lanes show steady-state levels of mRNA encoding MnSOD, IL-6, PAI-1, β_2-microglobulin, and c-*fos* after a 3-h treatment period in the presence of 10 μg of cycloheximide (CHX) per ml: control (C), 100 ng of muTNF per ml, 100 ng of hTNF per ml. The right three lanes show steady-state mRNA levels after a 12-h treatment period in the absence of cycloheximide: control (C), 100 ng of muTNF per ml, 100 ng of hTNF per ml.

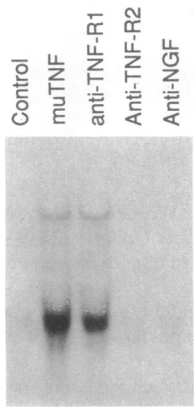

FIG. 5. Northern analysis of MnSOD mRNA in murine NIH 3T3 cells. Cells were treated with the indicated reagents for 12 h: control, 100 ng of muTNF per ml, 1:100 dilution of anti-TNF-R1 serum, 1:100 dilution of anti-TNF-R2 serum, 1:100 dilution of anti-NGF serum.

heximide. These results suggested that MnSOD induction is signaled through TNF-R1. To test this prediction, NIH 3T3 cells were treated with the agonistic anti-TNF-R1 and anti-TNF-R2 antibodies. As shown in Fig. 5, anti-TNF-R1 antibodies strongly induced MnSOD mRNA, whereas anti-TNF-R2 antibodies had no effect. These results demonstrate that the receptor responsible for signaling cytotoxicity (TNF-R1) also mediates the induction of a key protective activity.

DISCUSSION

We have previously shown that murine TNF-R1 has a similar affinity for muTNF and hTNF, whereas murine TNF-R2 is specific for muTNF (20). The murine system therefore allows predictions to be made as to which TNF receptor mediates a given response: TNF-R1 responses should be induced by muTNF and hTNF, whereas TNF-R2 responses should only be induced by muTNF. To validate this model and more directly examine the individual roles of the two TNF receptors, we generated rabbit polyclonal antibodies against soluble forms of both muTNF receptors.

Polyclonal antibodies directed against TNF-R2 were found to stimulate proliferation of murine thymocytes and the cytotoxic T-cell line CT6. However, polyclonal antibodies directed against TNF-R1 had no such proliferative effect. These results are consistent with the proliferation of these cell types in response to muTNF, and not hTNF (25, 28–30), which is also suggestive of a TNF-R2 response.

Cytotoxicity in murine LM cells is standardly used as a sensitive assay for hTNF (31). Also, LM cells exhibit a similar sensitivity to muTNF and hTNF (30). This is suggestive of a response mediated by TNF-R1 with little if any requirement for the binding of TNF to TNF-R2. In agreement with this, polyclonal antibodies directed against TNF-R1 induced cytotoxicity in LM cells, even at a serum dilution of $1:10^4$. No cytotoxicity was seen with TNF-R2 antibodies, despite the ability of these antibodies to behave as agonists in the T-cell proliferation assays. It is interesting to note that induction of the mRNA encoding the defense activity MnSOD was also TNF-R1 specific. Overexpression of MnSOD mRNA has been previously shown to counteract the cytotoxic effects of TNF, and therefore mitochondrial generation of O_2^- has been implicated as a key component of TNF-mediated cell killing (27). Thus, it appears that the cascade of events that lead to generation of O_2^- and the induction of a O_2^- scavaging activity are signaled by the same TNF receptor.

The inability of the TNF-R2 antibodies to act as agonists in the LM cytotoxicity assay was not due to the absence of TNF-R2 on LM cells; we previously showed that the TNF receptors expressed on the surface of LM cells are about 60% TNF-R2 and 40% TNF-R1 (20). In addition, the NIH 3T3 cells used in the MnSOD induction experiments expressed transcripts corresponding to TNF-R1 and TNF-R2 (data not shown). The results of the cytotoxicity and MnSOD mRNA studies therefore suggest that the functions of TNF-R1 and TNF-R2 are not redundant, but rather that only TNF-R1 signals these two responses.

A similar argument can be made for the different behavior of the two antibody preparations in the T-cell and thymocyte proliferation assays. Whereas CT6 cells express little or no TNF-R1 mRNA and do not bind detectable amounts of hTNF (20), thymus cells express mRNA for both receptor types (20) and the thymocytes used in the proliferation assays bind muTNF and hTNF (29) (indicative of the presence of TNF-R1). Therefore, it would appear that the proliferative signal for these cells can be mediated by TNF-R2 but not by TNF-R1. These results indicate that different TNF receptors signal cytotoxicity and T-cell proliferation and that the two TNF receptors are not redundant in signaling these functions.

The amount of primary sequence similarity between TNF-R1 and TNF-R2 is also suggestive of distinct functions for the two TNF receptors. For although the extracellular ligand-binding domains of the two TNF receptors show some homology (≈20%), their intracellular domains show no significant similarities (20). This would be consistent with the intracellular regions of the two receptors being coupled to different signal transduction pathways.

Several reports have described antibodies directed against human TNF-R1 that have agonist properties (15, 21, 22). These studies demonstrate that a specific conformational change induced by the TNF molecule itself is probably not responsible for the activation of TNF-R1. Rather, a nonspecific perturbation, most likely receptor dimerization or aggregation, can be sufficient to signal through this receptor. In this report, we show that TNF-R2 can also be activated by immunoglobulins in the absence of TNF. Therefore, signaling via TNF-R2 does not have an absolute requirement for TNF and may be initiated through a mechanism similar to that utilized by TNF-R1.

The absence of reports on TNF-R2 agonists may be a result of direct TNF-R2 responses being much less numerous than TNF-R1 responses. In support of this, most activity comparisons of hTNF and muTNF in mice or murine cell lines show relatively small differences, with T-cell and thymocyte proliferation being the most dramatic exceptions. Why TNF-R2 effects would be specific for such a small cell population is not clear, given the near ubiquitous distribution of this receptor in cell types and tissues. Perhaps a number of TNF-R2 responses are yet to be identified. Also, TNF-R2 may play an accessory role in mediating other TNF effects, even if it is not responsible for the signal transmission.

The experiments described in this study show that TNF-R1 can initiate signals for cytotoxicity and TNF-R2 can initiate signals for thymocyte and cytotoxic T-cell proliferation. However, it should be noted that TNF-induced proliferation may not be mediated by TNF-R2 in all cell types. Engelmann *et al.* (15) have shown that polyclonal antibodies against human TNF-R1 can stimulate proliferation of human FS11 fibroblasts. Thus, it appears that different TNF receptors can signal proliferation in different cell types. It is therefore likely that many additional studies will be required before a thorough understanding of the individual roles of the two TNF receptors will be realized.

We thank Bill Kohr and Helga Raab for help and advice on the purification of the soluble TNF receptors and also Greg Bennett and Roderick Vitangcol for preparation of the polyclonal antisera.

1. Decker, T., Lohmann-Matthes, M. L. & Gifford, G. E. (1987) *J. Immunol.* **138,** 957–962.
2. Turner, M., Londei, M. & Feldmann, M. (1987) *Eur. J. Immunol.* **17,** 1807–1814.
3. Spriggs, D. R., Imamura, K., Rodriguez, C., Sariban, E. & Kufe, D. W. (1988) *J. Clin. Invest.* **81,** 455–460.
4. Carswell, E. A., Old, L. J., Kassel, R. L., Green, S., Fiore, N. & Williamson, B. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3666–3670.
5. Sugarman, B. J., Aggarwal, B. B., Hass, P. E., Figari, I. S., Palladino, M. A. & Shepard, H. M. (1985) *Science* **230,** 943–945.
6. Goeddel, D. V., Aggarwal, B. B., Gray, P. W., Leung, D. W., Nedwin, G. E., Palladino, M. A., Patton, J. S., Pennica, D., Shepard, H. M., Sugarman, B. J. & Wong, G. H. W. (1986) *Cold Spring Harbor Symp. Quant. Biol.* **51,** 597–609.
7. Beutler, B. & Cerami, A. (1988) *Annu. Rev. Biochem.* **57,** 505–518.
8. Old, L. J. (1988) *Sci. Am.* **258**(5), 59–75.
9. Kull, F. C., Jacobs, S. & Cuatrecasas, P. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 5756–5760.
10. Baglioni, C., McCandless, S., Tavernier, J. & Fiers, W. (1985) *J. Biol. Chem.* **260,** 13395–13397.
11. Creasey, A. A., Yamamoto, R. & Vitt, C. R. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3293–3297.
12. Tsujimoto, M., Yip, Y. K. & Vilček, J. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7626–7630.
13. Hohmann, H. P., Remy, R., Brockhaus, M. & van Loon, A. P. G. M. (1989) *J. Biol. Chem.* **264,** 14927–14934.
14. Engelmann, H., Novick, D. & Wallach, D. (1990) *J. Biol. Chem.* **265,** 1531–1536.
15. Engelmann, H., Holtmann, H., Brakebush, C., Avni, Y. S., Sarov, I., Nophar, Y., Hadas, E., Leitner, O. & Wallach, D. (1990) *J. Biol. Chem.* **265,** 14497–14504.
16. Brockhaus, M., Schoenfeld, H. J., Schlaeger, E. J., Hunziker, W., Lesslauer, W. & Loetscher, H. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 3127–3131.
17. Schall, T. J., Lewis, M., Koller, K. J., Lee, A., Rice, G. C., Wong, G. H. W., Gatanaga, T., Granger, G. A., Lentz, R., Raab, H., Kohr, W. J. & Goeddel, D. V. (1990) *Cell* **61,** 361–370.
18. Loetscher, H., Pan, Y. C. E., Lahm, H. W., Gentz, R., Brockhaus, M., Tabuchi, H. & Lesslauer, W. (1990) *Cell* **61,** 351–359.
19. Smith, C. A., Davis, T., Anderson, D., Solam, L., Beckmann, M. P., Jerzy, R., Dower, S. K., Cosman, D. & Goodwin, R. G. (1990) *Science* **248,** 1019–1023.
20. Lewis, M., Tartaglia, L. A., Lee, A., Bennett, G. L., Rice, G. C., Wong, G. H. W., Chen, E. Y. & Goeddel, D. V. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 2830–2834.
21. Espevik, T., Brockhaus, M., Loetscher, H., Nonstad, U. & Shalaby, R. (1990) *J. Exp. Med.* **171,** 415–426.
22. Shalaby, M. R., Sundan, A., Loetscher, H., Brockhaus, M., Lesslauer, W. & Espevik, T. (1990) *J. Exp. Med.* **172,** 1517–1520.
23. Naume, B., Shalaby, R., Lesslauer, W. & Espevik, T. (1991) *J. Immunol.* **146,** 3045–3048.
24. Hohmann, H. P., Brockhaus, M., Baeuerle, P. A., Remy, R., Kolbeck, R. & van Loon, A. P. G. M. (1990) *J. Biol. Chem.* **265,** 22409–22417.
25. Ranges, G. E., Bombara, M. P., Aiyer, R. A., Rice, G. C. & Palladino, M. A. (1989) *J. Immunol.* **142,** 1203–1208.
26. Wong, G. H. W. & Goeddel, D. V. (1988) *Science* **242,** 941–944.
27. Wong, G. H. W., Elwell, J. H., Oberley, L. W. & Goeddel, D. V. (1989) *Cell* **58,** 923–931.
28. Ehrke, M. J., Ho, R. L. X. & Hori, K. (1988) *Cancer Immunol. Immunother.* **27,** 103–108.
29. Ranges, G. E., Zlotnik, A., Espevik, T., Dinarello, C. A., Cerami, A. & Palladino, M. A. (1988) *J. Exp. Med.* **167,** 1472–1478.
30. Rice, G. C., Kramer, S. M., Figari, I. S., Ranges, G. E. & Palladino, M. A. (1990) in *Tumor Necrosis Factor: Structure, Mechanism of Action, Role in Disease and Therapy*, eds. Bonavida B. & Granger, G. (Karger, Basel), pp. 87–93.
31. Kramer, S. M. & Carver, M. E. (1986) *J. Immunol. Methods* **93,** 201–206.