Distinct Roles of the Two Tumor Necrosis Factor (TNF) Receptors in Modulating TNF and Lymphotoxin α Effects*

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The role for the two tumor necrosis factor (TNF) receptors in discriminating TNF and lymphotoxin α (LTα) effects has been studied. TNF and LTα were equally mitogenic in F54 fibroblasts, which express a high amount of the p55 compared to the p75 TNF receptors (TNFRs). In contrast, TNF was more potent than LTα in mediating gene regulation and cytotoxicity in SW480-βGal cells and KYM-1 cells, which have a high p75/p55 TNFR ratio. Both TNF and LTα showed comparable affinities for the two TNFRs. However, in contrast to LTα, TNF dissociated rapidly from the p75 TNFR, whereas both cytokines dissociated slowly from the p55 TNFR. Soluble p55 TNFR was much more potent than soluble p75 TNFR in inhibiting TNF cytotoxicity, whereas both soluble receptors moderately decreased LTα-mediated cytotoxicity with comparable efficacy. Antagonistic monoclonal antibodies against either TNFR types markedly inhibited TNF effects. However, only the p55 TNFR antagonistic antibody significantly decreased LTα-mediated cytotoxicity and cytomegalovirus promoter activation, whereas blocking of the p75TNFR enhanced the LTα effects. These data suggest that whereas the p75 TNFR can both directly propagate TNF signals and "pass" TNF to the p55 TNFR, it attenuates LTα and may serve as a decoy receptor for this cytokine.

Tumor necrosis factor α (TNF)† and lymphotoxin α (LTα, LTβ-R) are pleiotropic cytokines which mediate a large variety of inflammatory, immunostimulatory, and antiviral responses (1). They are both members of the TNF ligand and receptor family, which now contains at least 12 ligand-receptor pairs (2). They are both members of the TNF ligand and receptor family, which now contains at least 12 ligand-receptor pairs (1). They are both members of the TNF ligand and receptor family, which now contains at least 12 ligand-receptor pairs (2).

†These abbreviations used are: TNF, tumor necrosis factor; LT, lymphotoxin; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazium bromide; CMV, cytomegalovirus; PBS, phosphate-buffered saline; BSA, bovine serum albumin.

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The specific roles of the two TNFRs in mediating the TNF and LTα signals are currently vividly debated (28, 29). In many cell types, studies with antagonistic antibodies have indicated that both receptors are important in mediating TNF effects (30). Furthermore, a unique "passing model" has been suggested to explain the role of the p75TNFR in mediating TNF responses (31). The basis for this model is that although TNF binds to the p75TNFR with somewhat higher affinity than to the p55TNFR, the rate of dissociation of TNF from the p75TNFR is higher than from the p55TNFR. This rapid rate of dissociation of the TNF-p75TNFR complex may facilitate interaction of TNF with the p55TNFR, suggesting a role of the p75TNFR in passing TNF to the p55TNFR, which has been postulated to be the main TNF signal transducer. On the other
TNF Receptors Discriminate between TNF and LTα Effects hand, also the p75 TNFR mediates several TNF effects such as proliferation of T- and B-cells (14, 32), activation of the human CMV promoter and induction of NF-κB (33), and cytotoxicity (8, 34, 35). Studies with agonistic antibodies specific for either of the receptors have indicated that at least in some cell types both receptors may induce similar effects, although possibly by using different intracellular signaling pathways (33–35). At present, the distinct functional roles of the two TNFRs, as well as their possible role in defining disparate TNF and LTα effects, are not clear.

Here we show that cells expressing a high proportion of the p75 TNFR are relatively resistant to the action of LTα and that LTα, in contrast to TNF, has a slow rate of dissociation from the p75 TNFR. Blocking of the p75 TNFR with antagonistic antibodies inhibited TNF effects but enhanced LTα activities in cells expressing a high p75/p55 TNFR ratio. It suggests a mechanism by which the high p75 TNFR expression may inhibit LTα effects and indicates distinct specific roles for the two receptor types in discriminating between TNF and LTα effects on cells.

EXPERIMENTAL PROCEDURES

Materials—Human recombinant TNF-α and TNF-β (LTα) were generously supplied by Genentech Inc. (San Francisco, CA) and had a specific activity of 7.6 × 10^6 units/mg and 10 × 10^6 units/mg, respectively, in the LM bioassay. Recombinant soluble TNF receptors were generously provided by Dr. Refaat Shalaby (Genentech Inc.) and were produced by transfecting insect cells with baculoviruses which contained genes encoding extracellular regions of the p55 and p75 TNFRs, respectively. The monoclonal antibodies 1A-1, which inhibits binding of TNF to the p75 TNFR, as well as htr-5 and htr-9, which inhibit binding of TNF to the p55 TNFR, have been described before (36–38). TNF and LTα were iodinated by the IODOGEN method (39) by incubating 10^9 cells in 1 ml of solid 1,3,4,5-tetrachloro-2-yl-2,5-diphenyltetrazolium bromide (MTT) for 8 h followed by dye extraction with isopropyl alcohol/HCl and measuring the absorbance of solubilized dye at 570 nm. Cell viability was calculated according to the formula: cell viability (% ) = (A570 control × 100)/A570 sample.

Binding of Labeled TNF and LTα—For measurements of specific binding of cytokines to cells, confluent cultures were incubated in 24-well tissue culture plates with the indicated amounts of iodinated cytokine in the absence or presence of 0.5 μM unlabeled cytokine or antibodies as indicated in PBS, 1 mg/ml BSA, 0.02% NaN3, solubilized in 0.5 ml/well 0.2% KOH and counted in a Gamma spectrometer (Packard, Meriden, IL). Cell-associated radioactivity in the presence of excess unlabeled cytokine was defined as nonspecific binding and was subtracted from the values obtained without cold cytokine present to obtain the specific binding. The nonspecific binding was generally less than 10% of the specific binding. In experiments to measure the rate of dissociation, cells as above were incubated with saturating concentrations of iodinated cytokines, both in the presence and absence of untr-1 and htr-9, as above. The medium was then removed and PBS/BSA/NaN3 containing 0.5 μM unlabeled cytokine was added. The cells were then incubated at room temperature for the indicated time periods, washed three times, and cell-associated radioactivity was assayed as described above. Shown are typical experiments out of at least three in each case.

Thymidine Incorporation Assay—Thymidine incorporation in Fs4 carcinoma cells was measured as described (30) by incubating 10^5 cells/well with cytokines or antibodies in 200 μl of CM as indicated in duplicate wells of 96-well flat-bottomed microtiter plates (Costar, Cambridge, MA) for 72 h at 37°C in a 5% CO2 atmosphere. 1 μCi/ml [methyl-3H] thymidine (Amersham International, Slough, UK) was added after 68 h, and the cells were harvested after 4 h with a Micromate 196 Cell Harvester (Packard). Incorporated thymidine was measured by counting the samples for 2 min in a Matrix 96 Direct Beta Counter (Packard).

Cytotoxicity Assay—The cytotoxicity response of WEHI clone 13 and KYM-1 cells was estimated as described earlier (24, 35) with small modifications. Briefly, KYM-1 cells (3 × 10^5 cells/well) were seeded into 96-well microtiter plates in 100 μl of CM and incubated overnight. The medium was replenished, and the cells were incubated with cytokines and antibodies as indicated in the presence of 0.5 μg/ml actinomycin D (Serva, Heidelberg, Germany) for 24 h at 37°C. Cell viability was then assayed by staining the cells with 0.5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) for 4 h followed by dye extraction with isopropyl alcohol/HCl and measuring the absorbance of solubilized dye at 570 nm. Cell viability was calculated according to the formula: cell viability (% ) = (A570 control × 100)/A570 sample, where A and B represent the A570 in control and cytokine-treated cells, respectively.

β-Galactosidase Assay—The activation of CMV promoter in the SW480-βGal cells was measured as described earlier (33). Briefly, cells were grown for 3 days at 37°C in microtiter wells (2 × 10^5 cells/well) of 96-well flat-bottomed plates. Then, cytokines and antibodies were added as indicated, and incubation was continued for 5 h at 37°C. Cells were washed in PBS, and 100 μl of 1.5 mM chlorophenol red-β-o-galactopyranoside (Boehringer Mannheim, Germany) in Hanks' balanced salt solution containing 0.5% Nonidet P-40 (Sigma) were added. Following incubation for 20 min at 37°C, the reaction was stopped by addition of 100 μl of 50 mM sodium carbonate (Sigma), and β-galactosidase activity was determined by measuring absorbance at 570 nm in a microplate reader (Bio-Rad Laboratories).

Stimulation of Cells, Preparation of Nuclear Extracts, and Electrophoretic Mobility Shift Assay—1 × 10^6 cells per well were grown in 6-well plates (Costar, Cambridge, MA) for 3 days at 37°C. Medium was replenished, and cells were stimulated with serial dilutions of TNF and LTα for 60 min (dose-response experiments) or with 10 ng/ml TNF and 100 ng/ml LTα (kinetic experiments). Nuclear extracts were prepared as described (33). Electrophoretic mobility shift assays were performed by incubating 1 μg of nuclear extract with 2 μg of poly(dI-dC) (Pharmacia Fine Chemicals, Uppsala, Sweden) in a binding buffer (20 mM HEPES, pH 7.9, 50 mM KCl, 1 mM EDTA, 1 mM DTT, 0.25 mM BSA, 100 μM dNTPs) in a total volume of 20 μl for 30 min at room temperature. End-labeled NF-κB-specific oligonucleotide probe (5′-AGTTGAGGGACTTTCCAGGG-3′) (Promega Corp., Madison, WI, 1 × 10^5 to 10^6 cpm) was added, and the mixture was incubated for another 10 min. The samples were separated on native 7% polyacrylamide gels (0.25 × Tris borate-EDTA; 100 V/h followed by 130 V/2-h). The gels were dried (80°C, 2 h) and exposed to x-ray film (X-Omat AR, Eastman Kodak).

RESULTS

The Relative Potency of TNF and LTα in Mediating Biological Responses—It is well known that in some cell lines TNF and LTα are equally potent in mediating a biological effect. Thus, TNF and LTα exhibited equal cytotoxic effect when added to WEHI clone 13 cells (Ref. 24 and data not shown). Furthermore, as shown in Fig. 1A, TNF and LTα were equally potent in inducing proliferation of the Fs4 human fibroblastic cell line. In contrast to this, LTα was less potent than TNF in other cell lines. Thus, as shown in Fig. 1B, about 10–50-fold higher concentrations of LTα compared to TNF were needed to obtain a similar cytotoxicity toward the human rhabdomyosarcoma cell line KYM-1, when applying the same preparations of the cytokines as used in Fig. 1A. Furthermore, when assaying for activation of a CMV reporter construct in the human adenocarcinoma cell line SW480-βGal, 500-1000-fold higher concentrations of LTα were required to obtain an effect similar to that
induced by TNF. Moreover, in SW480-βGal cells, the maximal response obtained with LTα was lower compared to TNF, and the dose of LTα, inducing the half-maximal response, was about 1000-fold higher than that for TNF (Fig. 1C). The same difference between TNF and LTα was seen when directly assaying activation of the transcription factor NF-κB in SW480-βGal cells, whereas the time required for the half-maximal response was indistinguishable between TNF and LTα (Fig. 2).

Fig. 1. Effects of TNF and LTα in Fs4 (A), KYM-1 (B), and SW480-βGal (C) cells. Cells were incubated as indicated with TNF (○) and LTα (●). Responses were assayed as described under “Experimental Procedures” and were: incorporation of [3H]thymidine in Fs4 cells, cytotoxicity measured as MTT release in KYM-1 cells, and activation of a β-galactosidase gene under the control of the CMV promoter in SW480-βGal cells.

Taken together, these results indicate that although TNF and LTα are about equally potent in inducing responses in some cell lines, other cells are more or less resistant to the action of LTα while still being susceptible to the action of TNF. The expression of TNF Receptors in Fs4, KYM-1, and SW480-βGal Cells—In order to understand the large relative differences in the sensitivity of the cell lines to TNF and LTα described above, we speculated that this could correlate with possible differences in binding of the two cytokines to these cell lines. The total number of receptors as well as the binding to each receptor were estimated by performing binding experiments in the presence and in the absence of antibodies which specifically block the binding of TNF and LTα to one but not the other receptor. As shown in Fig. 3, both TNF and LTα bound specifically and in a saturable manner to these cell lines. When the binding experiments were performed in the presence of the p75 TNFR blocking antibody, utr-1, the binding of both TNF and LTα was reduced by 10–20% in Fs4 cells, by 80–90% in KYM-1 cells, and by more than 95% in SW480-βGal cells. The p55 TNFR antibody htr-9 inhibited the binding of both TNF and LTα to Fs4, KYM-1, and SW480-βGal cells by 90%, 10%, and 5%, respectively. These data demonstrate that the binding sites recognized by both TNF and LTα are mainly of the p55 type in Fs4 cells, whereas in KYM-1 and SW480-βGal cells, 90% and 95%, respectively, of the binding sites are of the p75 type.

Scatchard analysis of the data indicated that all three cell lines used here express approximately similar numbers of the p55 TNFR per cell (1000–3000 receptors/cell). On the other hand, these cell lines express in the range from a few hundreds (Fs4) to more than one hundred thousands (SW480-βGal) of the p75 TNFR per cell (data not shown). Taken together, these results indicate that high p55/p75 TNFR ratio, whereas the specific activity of TNF is not particularly affected by the p55/p75 ratio. It should also be noted that in all three cell lines examined, the sum of the binding of both TNF and LTα in the presence of utr-1 and htr-9 equals the specific binding in the absence of antibodies. Thus, there is no indication of the existence of a third type of binding sites for either TNF, or LTα, which could explain the differences in their biological activity.

Affinity of TNF and LTα Binding to the p55 and p75 TNFR—It has been shown previously that TNF binds to the p75 TNFR with a dissociation constant of 0.3–0.5 nM, whereas the dissociation constant for TNF binding to the p55 TNFR has been reported to be 3–5 times higher (0.7–1.5 nM) (31, 34). Here we show Scatchard analysis of the
binding of LTα toward the Fs4 and KYM-1 cells, both in the absence and in the presence of the antibodies utr-1 and htr-9 (Fig. 4, A and B). These data demonstrate that LTα binds also to the two TNFRs in a way comparable with TNF. Thus, for LTα, we estimated \( K_d \) values of 0.3 nM and 0.6 nM for the p75 and the p55 TNFRs, respectively. Furthermore, as in the case of TNF, LTα appeared to bind to both the p55 and the p75 TNFRs with comparable affinity constants in Fs4 and KYM-1 cells (data not shown).

Taken together, these data indicate that the difference in specific activity between TNF and LTα in Fs4 and KYM-1 cells cannot be explained by the differences in affinities of LTα and TNF to the p55 and p75 TNFRs.

Differences in the Rate of Dissociation of TNF and LTα from the p75 TNFR—The equilibrium binding experiments described above revealed no major differences between TNF and LTα which could explain the large differences in biological activity between the cell lines. To further examine the binding of these cytokines to their receptors, we therefore studied the dynamic interactions between receptors and ligands by measurements of the rates of dissociation.

As shown in Fig. 5A, TNF rapidly dissociated from the p75 TNFR cells. In striking contrast to this, LTα appeared to have a relatively slow rate of dissociation from this receptor, indicating that the on-off rate of the LTα-p75 complex is much slower than the on-off rate of the TNF-p75 complex. The experiments shown were performed at room temperature and indicate at least a 6-fold difference between TNF and LTα in the rate of dissociation from the p75 TNFR at this temperature (\( t_{1/2} \sim 5 \) min for TNF versus more than 30 min for LTα). The rate of dissociation for LTα and TNF from the p55 TNFR appeared to be indistinguishable, and somewhat lower (\( t_{1/2} > 60 \) min) than the rate of dissociation for LTα from the p75 TNFR (Fig. 5B). These data demonstrate that there are major differences between TNF and LTα in their interaction with the p75 TNFR, whereas the experiments reveal no differences in the interaction with the p55 TNFR.

Effect of Soluble TNFRs on TNF and LTα Activity—At least two predictions can be made on the basis of the results described above. First, soluble p55 TNFR may be more potent than soluble p75 TNFR in inhibiting TNF effects, and, second, both receptors could be equally potent in decreasing LTα activity. To test this, murine WEHI clone 13 fibroblasts were incubated with saturating amounts of iodinated TNF and LTα as above. Cells were washed, and unlabeled TNF and LTα were added to wells containing bound labeled TNF and LTα, respectively. Cell-associated radioactivity was measured at the indicated time points thereafter as described under “Experimental Procedures.”

As shown in Fig. 6, inhibition of TNF cytotoxicity by the p55 TNFR was more pronounced than the inhibition of TNF by the p75 TNFR, whereas the experiments reveal no differences in the interaction with the p55 TNFR. Effect of Soluble TNFRs on TNF and LTα Activity—To test functional consequences of the difference in the rate of dissociation for TNF and LTα from the p75 TNFR, we applied antagonistic p55 and p75 TNFR monoclonal antibodies (htr-5 and utr-1, respectively). As shown in Fig. 7, A and B, both htr-5 and utr-1 markedly inhibited the TNF-induced CMV promoter activation in SW480-βGal cells and KYM-1 cytosis. In contrast, only treatment of cells with htr-5 de-
increased the ability of LTα to mediate biological responses. Interestingly, blocking of the p75 TNFR with utr-1 enhanced the LTα-mediated activation of the reporter construct and its cytotoxic effect (Fig. 7, B and D). These data show that TNF uses both TNFR types for mediating signal transduction, whereas LTα involves only the p55 TNFR. In addition, these results indicate that blocking of the p75 TNFR inhibits the TNF-mediated responses but increases the LTα activities in cells expressing a high p75/p55 TNFR ratio.

DISCUSSION

Why TNF and LTα show, depending on the cell type, either similar or different biological effects remains one of the unresolved questions in the cytokine field. The present paper demonstrates a correlation of cell responsiveness to TNF and LTα with the expression of the p75 TNFR. Thus, TNF was 100-1000-fold more potent than LTα in cells expressing a high p75/p55 TNFR ratio, whereas both cytokines showed equal efficacy in evoking biological responses of cells expressing largely the p55 TNFR. The correlation of expression of a high proportion of the p75 TNFR with the relative resistance of cells to LTα is not restricted to the three cell lines described here. Also the human myeloma cell line OH-2, which predominantly expresses the p75 TNFR, is 100-100-fold more resistant to the growth stimulatory action of LTα compared with TNF. In contrast, in U937 cells, which express about 80% p75 and 20% p55 TNFRs (41, 42), LTα is required in at least 10-fold higher concentrations than TNF to obtain a similar cytotoxic effect (41). These data, together with the results on the lack of homology in the receptor binding regions of TNF and LTα (4), their differences in trimer formation (43), and stability of trimers (44), suggest different modes of receptor interactions and/or receptor triggering caused by TNF and LTα.

To verify this hypothesis, we first studied the interaction of TNF and LTα with the two TNFRs by measuring their affinities and on-off rates of dissociation. Comparable Kd values have been detected for both cytokines upon their binding to the two TNFRs. Together with previously published data (41, 45), it suggests that different efficacy of TNF and LTα cannot be explained by their different affinities for the TNFRs. Of importance, differences between TNF and LTα in the dynamic interaction with the receptors have been observed, which are not reflected in the measurements of affinity constants. Thus, TNF had a much more rapid on-off rate in the interaction with the p75 TNFR than with the p55 TNFR. In contrast, LTα showed only minor differences in its rate of dissociation from the p75 TNFR compared with that from the p55 TNFR and exhibited a significantly slower rate of dissociation from the p75 TNFR than TNF.

Secondly, the aforementioned difference between TNF and LTα in the dynamic interaction with the p75 TNFR has been confirmed by using soluble TNFRs. In accordance with the data reported by other groups (17, 18), the soluble p55 TNFR was considerably more potent than the soluble p75 TNFR in inhibiting a TNF effect. Assuming that a rapid on-off rate of TNF in the interaction with soluble receptors increases the probability of interaction of TNF with a signal transducing cell surface receptor, the difference reported here can be explained by the differences in the rates of dissociation for TNF between the p55 and p75 TNFR. Also, in the case of LTα, similar rates of dissociation in the interaction with the two TNFRs are reflected in similar abilities of the two soluble receptors in inhibiting the LTα cytotoxic effect. Interestingly, however, the inhibition of the LTα effect by either TNFR type was much less pronounced than the inhibition of the TNF activity by the p55 TNFR. These results confirm the finding that LTα has a lower potency to bind to the soluble form of the p55 TNFR when compared to TNF (46). Furthermore, in attempts to attenuate cytokine action in vivo, the implication of these results could be that in the case of TNF soluble p55 TNFR should be a more potent antagonist than the p75 TNFR, whereas none of the soluble receptors may be strong antagonists of LTα action in vivo.

Thirdly, the application of antagonistic TNFR antibodies in this study has revealed the different use of the p75 TNFR by TNF and LTα. Blocking of both the p55 and p75 TNFRs led to a marked inhibition of the TNF effects in SW480-βGal and KYM-1 cells. In accordance with previously published observations (6, 8, 36, 41), it indicates that TNF uses both TNFR types for signaling. In contrast, the antagonistic p75 TNFR antibody utr-1 potentiated the ability of LTα to mediate CMV promoter activation in SW480-βGal cells and to cause cytotoxic effect in KYM-1 cells, whereas blocking of the p55 TNFR inhibited the LTα effects. In line with this, only the p55 TNFR has been reported to mediate the LTα cytotoxic effect in U937 cells (41) and its ability to up-regulate the expression of adhesion molecules in human vascular endothelial cells (47) as well as the proliferative response of human primary fibroblasts (44), whereas both receptors were important for the TNF effects (36, 41). Thus, despite the ability of LTα to bind with high affinity to the p75 TNFR (28), at least in some cell lines, this cytokine does not seem to mediate signal transduction through this receptor type.

The p75 TNFR has also been proposed to play an accessory function by passing TNF to the p55 TNFR due to its higher affinity, thereby providing more efficient signal transduction by the p55 TNFR (28). However, it is unlikely that the p75 TNFR mediates interaction of LTα with the p55 TNFR in SW480-βGal and KYM-1 cells, because in this case the on-off rate apparently is much slower. On the opposite, it could attenuate LTα and prevent its binding to the p55 TNFR. Indeed, antibodies which block the binding of LTα to the p75 TNFR without affecting the binding to the p55 TNFR potentiated the LTα effects but inhibited the TNF activities in SW480-βGal and KYM-1 cells. Thus, whereas the p75 TNFR is capable of both directly propagating TNF signals and “passing” TNF to

2 Borset, M., Medvedev, A. E., Sundan, A., and Espevik, T. (1996) Cytokine, in press.
3 A. E. Medvedev, T. Espevik, G. Ranges, and A. Sundan, unpublished results.
the p55 TNFR, it appears to attenuate LTα and to serve as a "decoy" receptor for this cytokine in SW480-βGal and KYM-1 cells.

Under normal physiological conditions, LTα forms a heteromeric complex with LTβ on the cell surface (9). It has been published that LTβ can be found only in a membrane-associated form (40). A novel LTβ specific receptor was identified, which binds to heterotrimers with the stoichiometry of LTβ2/LTα1 but not to those with the stoichiometry LTβ1/LTα2 (10). Moreover, LTα/LTβ complexes with different stoichiometries can be distinguished by the LTβR and the p55 TNFR (40). To the best of our knowledge, no binding between the LTβR and the LTα homotrimers has been reported. In addition, radiolabeled TNF can be displaced with excess cold LTα and vice versa, and the combination of the p55 and p75 TNFR antagonistic antibodies completely prevented the binding of both cytokines to the cell lines.3 Thus, the binding data obtained in this study with soluble LTα give no indication of the involvement of LTβ or the LTβ specific receptor in the binding of homotrimeric LTα to the cell lines examined.

Interestingly, TNF and LTα exhibited comparable abilities to inhibit proliferation of the human breast carcinoma cell line BT-20, that had only low affinity receptors (suggesting the p55 TNFR), whereas in cell lines that had high affinity receptors (suggesting the p75 TNFR), TNF was 20 to 320 time more potent than LTα (45). It is plausible that the lack of LTα signaling through the p75 TNFR and the ability of this receptor to attenuate LTα, found in the present study, could be the reasons explaining differential biological effects of these cytokines. In contrast, LTα, but not TNF, acts as a growth factor for Epstein-Barr virus-infected B cell lines which express mainly the p75 TNFR (26). The reason for this discrepancy is not clear but could be related to the importance of the two TNFRs in mediating specific biological responses in various cell types. Further studies of the role of the two TNFRs in TNF and LTα signaling, especially in the cell types where the p75 TNFR is the main signal transducing molecule, are needed to bring a better understanding of the mechanisms behind differential or similar effects of these two cytokines.

4 A. E. Medvedev, T. Espevik, G. Ranges, and A. Sundan, unpublished observation.
TNF Receptors Discriminate between TNF and LTα Effects

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