Interaction between the mRNA of the 55-kDa Tumor Necrosis Factor Receptor and Cellular Proteins

POSSIBLE INVOLVEMENT IN POST-TRANSCRIPTIONAL REGULATION OF RECEPTOR EXPRESSION*

(Received for publication, December 11, 1995, and in revised form, March 4, 1996)

Reinhard Winzent, Sabine Kafert, Bettina Preiß, Heidrun A. Mylius-Spencker, Klaus Resch, and Helmut Holtmann§

From the Institute of Molecular Pharmacology, Medical School, D-30623 Hannover, Federal Republic of Germany

Numerous effects of tumor necrosis factor are signaled by its 55-kDa receptors. Studying their expression, we found that the level of receptor mRNA was decreased during the phorbol ester-induced differentiation of myelomonocytic cell lines. While only minor changes in transcription were noted, the half-life of receptor mRNA in the differentiated cells was markedly decreased, indicating the involvement of post-transcriptional regulation. In an electrophoretic mobility shift assay, formation of complexes between radiolabeled receptor mRNA and cellular proteins was observed. The decrease in receptor mRNA levels during phorbol ester-induced differentiation was paralleled by a change in the pattern of these complexes. Protein-RNA interaction was selective, as it was not competed by unrelated RNAs. Yet, certain mRNAs that contain AU-rich sequences, known to be involved in the control of their stability, did compete with the receptor mRNA, although the latter is devoid of such sequences. A region of 18 nucleotides within its coding region was found to contain an element essential for the formation of all complexes and sufficient for the formation of those with lower molecular mass. Adjacent bases were required in addition for the formation of the complexes with higher molecular mass. The results suggest that proteins interacting with this region of the 55-kDa tumor necrosis factor receptor mRNA contribute to the regulation of its expression.

Tumor necrosis factor (TNF), an inflammatory cytokine primarily produced by activated macrophages, exerts a wide range of biological activities. TNF affects the growth, differentiation, and function of many cells involved in immune and inflammatory processes (1). Two distinct cell surface receptor molecules with molecular masses of approximately 55 kDa (2-4) and 75 kDa (5, 6) have been identified and cloned. They are expressed independently of each other in varying proportions, depending on the cell type (7, 8). The 55-kDa receptor species (TNFR-55) has been found to signal for most cellular effects of TNF, including growth stimulation as well as cytotoxicity (9, 10). Mice in which expression of this receptor species was abolished were reported to be more sensitive to certain types of infection and, on the other hand, to exhibit increased resistance to endotoxin-induced lethality (11, 12). These observations underscore the contrasting nature of consequences of the response to TNF noted earlier (13), consequences that are important for the defense against pathogens, but can damage the host as well. Since the TNFR-55 is crucial also for the adverse effects of TNF, the possibly deleterious function of this receptor species requires mechanisms that stringently control its activity. Besides regulation of TNF production (reviewed in Ref. 1) and of its availability (through soluble forms of the receptors, Ref. 4), also responsiveness of the target cells is controlled, both at the level of signaling as well as through modulation of receptor expression (14, 15).

The number of TNFR-55 molecules on the surface of a wide variety of cells is low, ranging between several hundred and a few thousand. In response to various stimuli the receptor number can be further down modulated, due to rapid internalization (15) and to shedding of their extracellular domains (16). Synthesis of the TNFR-55 is subject to modulation as well. Increased numbers of receptors on the cell surface have been noted in various cells in response to IFN-γ (17). In keratinocytes exposed to UV radiation or to TNF, the amount of TNFR-55 and of its mRNA is initially decreased and later on increased (18). Decreased amounts of cell surface TNFR-55, corresponding to decreased mRNA levels, have been observed by us in cells of the promyelocytic line HL-60 following their differentiation into macrophage-like cells (19). As shown in the present study, the decrease in mRNA for the p55 receptors appears to involve a decrease in its stability.

The control of mRNA stability has been recognized as an important mode of regulation of gene expression (20-23). Examples of this type of regulation are represented by the mechanisms that affect degradation of the transcripts for the transferrin receptor (24), β-tubulin (25), and histone (26). The short half-lives of a number of proto-oncogene and lymphokine gene encoded mRNAs apparently depend on AU-rich elements (ARE) (27, 28), especially on reiterations of the pentamer (ARE) (27, 28), especially on reiterations of the pentamer (ARE) (27, 28), especially on reiterations of the pentamer (ARE) (27, 28), especially on reiterations of the pentamer (ARE) (27, 28).

In accordance with biological activities.
the ARE-containing 3'-UTR were deleted (34). The mRNAs for granulocyte-macrophage colony-stimulating factor (GM-CSF) (35) and ribonucleotide reductase R1 and R2 (36, 37) contain sequences within the 3'-UTR that are involved in alterations of message stability in response to phorbol esters.

A common denominator of this type of regulation appears to be the interaction of specific trans-acting proteins with the cis-acting elements in the mRNA molecules. Thus, the iron response element in the transferrin receptor binds to a specific cytoplasmic protein (38, 39). Phorbol esters decrease the activity of cytoplasmic protein to form a 57-kDa complex with ribonucleotide reductase R1 mRNA (36) and a 45-kDa complex with ribonucleotide reductase R2 mRNA (37). Several ARE-binding proteins have been identified (e.g. Refs. 40–42) and some have been shown recently (43–45). These (putative) trans-acting factors are likely to function as critical regulators of mRNA stability.

Here we report that the decrease in stability of the TNFR-55 transcripts during differentiation of myelomonocytic cell lines is associated with changes in the interaction of one or several proteins with this mRNA. Localization of the region to which the protein(s) bind suggests that control of TNFR-55 mRNA degradation involves a novel cis-acting element in its coding region.

MATERIALS AND METHODS

Cell Culture—The human cell lines U-937 (histiocytic lymphoma), HL-60 (promyelocytic leukemia), and THP-1 (monocytic leukemia) were obtained from the American Type Culture Collection and grown in RPMI 1640 medium supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), and 5% fetal calf serum. Dulbecco's modified Eagle's minimal essential medium supplemented in the same way was used for culturing the human fibroblastoid cell line SV-80 (46).

Preparation of Cytoplasmic Extracts—Cytoplasmic extracts of U-937, HL-60, and THP-1 cells into isoelectric focusing (IEF) gels was induced by incubating them with the phosphor ester 12-O-tetradecanoylphorbol-13-acetate (TPA) (5 nm) for 48 h (for details see Ref. 19).

Detection of TNFR-55 mRNA—For Northern blot analysis, total RNA was purified, electrophoresed in 1% agarose gels (6.8% [w/v] formaldehyde), transferred, and cross-linked to nylon membranes (Hybond N, Amersham-Buchler). Prehybridization and hybridization with a radio-labeled TNFR-55 cDNA probe, prepared by random primed labeling (Megaprime DNA labeling system, Amersham-Buchler), washing of the membranes, autoradiography, and laser densitometric quantitation were carried out essentially as described by Groudine (48).

Transcriptional Analysis—Nuclear run-on experiments were carried out essentially as described by Groudine (48). Following cultivation for 48 h in the absence or presence of TPA (5 nm), cells (10⁶ per sample) were lysed, and the nuclei were isolated and incubated with 200 μCi of [α-32P]UTP (800 Ci/mmol, Hartmann Analytic) and unlabeled UTP were included in the reaction mixture at a final concentration of 24 μM. Following phenol/chloroform extraction, transcription mixtures were passed through a NucTrap push column (Stratagene) to remove free nucleotides, and stored at −80 °C. Specific activity was typically 10⁵–10⁶ cpm/μg of RNA.

Generation of RNA Fragments by RNase H Digestion—For the generation of different short parts of a labeled RNA fragment (nt 343–423), 6 pmol of the fragment were hybridized with antisense oligonucleotides (0.3 nmol each) in 25 μl of 1× EDTA, pH 8.0, containing 20 μg of tRNA for 10 min at 25 °C. Further incubations were performed according to Mercer and Wake (51) with modifications. Briefly, KCl was added to the reaction to a final concentration of 50 mM, and hybridization was continued for 10 min at 25 °C. Thereafter, the solution was added with 20 μl Tris-HCl, 8.0, 50 mM KCl, 1 mM dithiothreitol, and 10 mM MgCl₂, and incubated at 37 °C with 1 unit of RNase H for 30 min and with 20 units of DNase 1 (RNase-free) for another 30 min. After phenol/chloroform extraction, precipitation, and reconstitution in water, the RNA was mixed with an equal volume of formamide, heated for 5 min at 56 °C, and electrophoresed on a denaturing polyacrylamide gel (15% acrylamide). After confirming that fragments of the expected size were generated (52, 53), they were excised from the gel and eluted with the crush and soak method (54). Identity of the fragments was confirmed by the pattern of RNA fragments obtained in digestions with RNases T1 (cleaving specifically after G) and T2 (cleaving preferentially after A in the presence of 3.5 μm uracil).

Detection of Protein-RNA Complexes in Electrophoretic Mobility Shift Assay—The radio-labeled RNA probe (1.5 × 10⁶ cpm, ~2 fmol) was incubated with cytoplasmic extracts (3 μg of protein/sample) in 25 μl Tris-HCl, pH 7.9, 15 mM KCl, 5 mM dithiothreitol, 0.5 mM EDTA, and 4 μg of RNA in a total volume of 20 μl for 10 min at 30 °C. The mixture was then incubated with RNase T1 (30 units/sample) for 30 min at 37 °C and electrophoresed on a nondenaturing polyacrylamide gel (5% acrylamide) in 0.25 × TBE (Tris borate-EDTA) buffer. In competition experiments the cytoplasmic extracts were incubated with the indicated amounts of unlabeled competitor RNA for 10 min prior to addition of the labeled RNA. In UV cross-linking studies, RNase T1 digestion was followed by exposure of the reaction mixtures to UV light on ice at 900 mJ/cm². Laemmli buffer (55) was then added, and the samples were passed through a 3% gel and analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) (12.5% acrylamide). The gels were fixed in 10% (v/v) trichloroacetic acid, dried, and autoradiographed at −70 °C with intensifying screens.

RESULTS

Decreased Stability of TNFR-55 mRNA upon Differentiation of Myelomonocytic Cell Lines Is Associated with a Change in the 
Formation of Protein-RNA Complexes—A decrease in the level of mRNA for the TNFR-55 upon TPA-induced differentiation, observed previously in the promyelocytic leukemia line HL-60 (19), was also noted in two other myelomonocytic lines, U-937 and THP-1 (Fig. 1). The amounts of TNFR-55 message were decreased by 66–87% in different experiments, without consistent differences between the cell lines.

To determine the mechanisms involved, the half-life of receptor mRNA in untreated and TPA-treated U-937 cells was compared. Actinomycin D was added to block transcription, and RNA was isolated from the cells at different times thereafter. As shown by Northern blot analysis (Fig. 2) the rate of degradation in cells treated with TPA was significantly enhanced (half-life ~ 1 h according to laser densitometric quantitation), compared with that of untreated U-937 cells (half-life ~ 3.5 h). Transcription of the TNFR-55 gene was estimated in nuclear run-on assays. The signal of the TNFR-55 transcripts had very low intensity (not shown), as compared with that of β-actin as a constitutive control and of c-fos as a control for TPA responsiveness (56). Quantitation by Phosphorlager imager revealed that, compared with untreated cells, transcription of the receptor gene in the TPA-treated cells was decreased only slightly (by 19 ± 12% in three experiments). Poor regulation of transcription is in accordance with sequence information on the promoters of the human and murine TNFR-55, showing that they bear characteristics of house keeping-type promoters (57, 58). Taken together, these observations indicated that TPA-induced down-regulation of TNFR-55 mRNA levels is largely due to increased degradation of the transcripts.

The regulation of mRNA stability appears to involve interaction of the mRNAs with distinct proteins (36–42). To detect proteins that bind to the TNFR-55 mRNA, electrophoretic mobility shift assays were performed employing radiolabeled full-length in vitro transcribed RNA. Incubation of the probe with cytoplasmic extracts from untreated U-937 cells resulted in formation of one major complex (Fig. 3, complex A) and one minor complex with lower electrophoretic mobility (complex B). A significantly different pattern was observed with protein extracts from TPA-treated U-937 cells: formation of complex A was decreased, whereas that of complex B was markedly increased. In addition, complexes of intermediate mobility appeared (bracket in Fig. 3). Formation of intermediate complexes varied in different experiments (compare eg. Fig. 4 and Fig. 7) for reasons not known at present.

The binding of proteins to the TNFR-55 RNA was specific, as demonstrated by the observation that unlabeled TNFR-55 RNA efficiently competed with the labeled transcripts in complex formation, whereas an unrelated transcript, of the neomycin resistance gene, did not compete (Fig. 3). Lack of competition was also observed for several other transcripts, including that of the 75-kDa TNF receptor (not shown) and various mRNA fragments (see Figs. 7B and 8A). The participation of proteins in complex formation was verified in experiments showing that the generation of bands with the mobility of complex A, B, or intermediate complexes (i) required the presence of cytoplasmic extracts and (ii) was sensitive to proteinase K treatment (not shown).

These results suggest that the TNFR-55 mRNA specifically interacts with cellular proteins. As shown in Fig. 4, the change in the activity of those RNA-binding proteins during TPA-induced differentiation occurs only gradually. The time course of the change in the binding pattern thus correlates closely to the time course of the change in the amounts of mRNA observed under these conditions (19).
Similar changes in the pattern of protein-RNA complexes, associated with TPA-induced differentiation, could be observed in extracts from all three myelomonocytic cell lines that exhibit the decrease in TNFR-55 mRNA (compare Fig. 5 with Fig. 1). In contrast, in the fibroblastoid cell line SV-80, TPA did not induce alterations in the pattern of protein-RNA complexes, nor did it affect the level of TNFR-55 cell surface expression nor the decrease in TNFR-55 mRNA (compare Fig. 5 with Fig. 1).

Initial Characterization of the Protein(s) Binding to the TNFR-55 mRNA—Protein-RNA complexes were cross-linked by UV radiation and subjected to SDS-PAGE to obtain initial information on the size of the protein(s) they contain (Fig. 6). The results were consistent with those obtained in the band shift experiments. Complex A, formed during interaction of the RNA with the extracts from untreated U-937 cells, corresponded to a cross-linked protein-RNA complex that migrated with a molecular mass of approximately 55 kDa. With the extracts from TPA-treated cells formation of that complex was decreased, whereas two additional complexes with molecular masses of 75–80 kDa were formed. Labeled RNA was displaced from the complexes by an excess of unlabeled TNFR-55 RNA, confirming the specificity of the interaction.

In a number of RNA species, AREs in their 3′-UTR have been identified as the target structure for RNA-binding proteins (40, 41, 43). The TNFR-55 mRNA lacks such sequences. Yet when examining whether the protein(s) interacting with the TNFR-55 mRNA might be related to ARE-binding proteins, it turned out that RNAs of GM-CSF, c-myc, and TNF, which contain AREs, effectively competed with labeled TNFR-55 RNA in formation of complex B (Fig. 7A). Competition was less pronounced in formation of complex A.

The c-myc RNA contains, in addition to the ARE, a target structure for protein binding in the C-terminal part of its coding region (59). In the murine GM-CSF transcript, an element in the 3′-UTR distinct from its ARE was identified, conferring responsiveness to regulation by TPA (35). To test which of the known target structures for protein binding was essential for the observed competition with the TNFR-55 RNA, fragments of the c-myc and GM-CSF RNAs that contained either the AREs or the target sequences mentioned above were compared in their ability to compete (Fig. 7B). Only the fragments containing the AREs were effective competitors, indicating that the TNFR-55 mRNA-binding protein(s) involved in formation of complex B can interact in some way with the AREs of GM-CSF and c-myc RNA.

Localization of the Sequence within the TNFR-55 mRNA Involved in Protein Binding—A series of fragments of the complete TNFR-55 mRNA was generated by in vitro transcription. Labeled or unlabeled RNA fragments were tested for protein binding or for competition with full-length TNFR-55 RNA, respectively (Fig. 8A). A labeled 81-nt fragment (containing nt 343–423) gave rise to protein-RNA complexes undistinguishable from those formed with the full-length TNFR-55 message. Furthermore, an excess of unlabeled 81-nt fragment competed out the TNFR-55 full-length RNA. Fragments lacking that region of the mRNA did not show complex formation or competition. Thus the nucleotide sequence involved in the formation of the protein-RNA complexes A, B, and intermediate complexes is located in the coding region, within nt 343–423 of the TNFR-55 mRNA.

For further localization of the protein binding region, we hybridized the full-length TNFR-55 RNA with antisense deoxynucleotides corresponding to different parts of the 81-nt fragment and tested their effect on the formation of protein-RNA complexes. As shown in Fig. 8B, an excess of antisense oligonucleotide E, complementary to nt 406–423, caused a significant reduction in the amounts of protein-RNA complexes.
The results presented in this and in a previous study (19) demonstrate that the steady state level of mRNA for the 55-kDa TNF receptor decreases during the phorbol ester-induced differentiation of myelomonocytic cell lines. These changes appear to be largely due to an increased rate of degradation of the mRNA in the differentiated cells (Fig. 2). The participation of post-transcriptional mechanisms in the control of the TNFR-55 mRNA level as observed here is in accordance with the information that the promoter of the TNFR-55 gene resembles that of housekeeping genes (57, 58). Furthermore, the murine TNFR-55 promoter has not been found regulated in experiments with promoter-reporter constructs (57).

In those instances where the analysis of mechanisms involved in the regulation of mRNA degradation has been approached, the transcripts have been demonstrated to interact with distinct cellular proteins (e.g. Refs. 36-45). As shown in Fig. 3, specific interaction with proteins is also observed for the TNFR-55 transcripts. The pattern of complexes formed between the TNFR-55 mRNA and proteins differs between untreated and TPA-treated cells. Both, in terms of kinetics and of cell specificity, the changes in the pattern of complexes closely correlate to the decrease in mRNA amounts (Figs. 4 and 5), suggesting that the proteins binding to the TNFR-55 mRNA take part in the regulation of its stability.

The nature of the proteins is not clear at present. Denaturing gel electrophoresis of the cross-linked complexes (Fig. 6) does not allow direct estimation of the size of the proteins, since the contribution of the RNA part to the size of the complex is not known. However, the observation that in the case of complex A a fragment of 18 nt (fragment I in Fig. 8C) yields a complex of the same mobility as the complete RNA puts 18 nt as an upper limit for RNA engaged in complex A. Applying the same considerations for complex B results in an upper limit of 33 nt (fragment II in Fig. 8C) present in that complex. Accordingly, the molecular masses of the protein component can be estimated to be at least 49 kDa in complex A and 64-69 kDa in complex B. We speculate that the TPA-induced change in the formation of complexes with rather extensive differences in size is caused by (i) replacement of the protein involved in complex A by a larger protein or by (ii) the binding of additional proteins to the RNA. Binding of an additional protein to the one already attached to the RNA in complex A is not likely: such a complex would be disrupted and thus remain undetected in SDS-PAGE, since protein-protein cross-linking by UV light is inefficient under the conditions chosen (61).

A number of transiently expressed mRNAs, including those of several cytokines and proto-oncogenes, contain AREs in their 3'-UTR, which have been shown to confer instability to the RNA (27, 28). The mechanism by which they function is not known in detail. It has been demonstrated, however, that the AREs interact with cytoplasmic proteins apparently involved in message degradation (62, 63). Despite the fact that the TNFR-55 mRNA does not contain such AREs, the RNAs for GM-CSF, TNF, and c-myc, which do contain AREs, efficiently compete with the TNFR-55 RNA in the formation of complex B. The regions containing the AREs appeared to be essential for competition (Fig. 7). Interestingly, competition in formation of complex A was much weaker, pointing to an important
difference between the two complexes. The observed competition indicates that the proteins that bind to the TNFR-55 mRNA are also able to bind AU-rich sequences. While complexes comparable in size with complex B have been noted (40), most of the ARE binding factors characterized so far give rise to complexes of 28–45 kDa (41, 43, 45, 64) and thus differ from the proteins described here. The relationship between the RNA-binding proteins reported in this work and the ARE-binding proteins needs to be clarified in further studies.

The sequence of the TNFR-55 mRNA involved in the formation of all complexes observed could be localized within the coding region. While in most cases of regulated mRNA degradation cis-acting elements are located in the 3' UTR, in some transcripts, e.g. of c-fos and c-myc, the coding region has been reported to contain instability elements as well (32, 33). The sequence of the TNFR-55 mRNA required for the formation of complexes with protein lies within an 18-nt region (nt 406–423, Fig. 8). It appears that this region is also sufficient to form complex A upon interaction with cytoplasmic extracts (Fig. 8C). For the formation of complex B and intermediate complexes, sequences within the next 15 nt 5' to this element are required. The fact that in the experiment shown in Fig. 8B the corresponding antisense oligonucleotide did not decrease complex formation indicates that only a few of the 15 nt, those adjacent to position 406, might be involved in protein binding. Using the FoldRNA program of the HUSAR/GCG package, no relevant secondary structures were detected in this region. No apparent sequence homologies to known cis-acting elements of other transcripts were noted.

In this study, TPA was used to induce differentiation of U-937 cells into macrophage-like cells. Phorbol esters like TPA, which activate protein kinase C (65), can modulate gene expression by affecting transcription (66, 67). There is also evidence that TPA affects gene regulation post-transcriptionally by altering the stability of mRNAs. Iwai et al. (35) could demonstrate that TPA stabilizes murine GM-CSF mRNA in EL-4 thymoma cells, and a 60-nucleotide sequence within the 3'-UTR was found essential for that effect. In TPA-treated mouse fibroblasts the increase in stability of ribonucleotide reductase R1 and R2 appears to involve decreased binding of a 52–57-kDa and a 45-kDa protein, respectively, to specific regions of the mRNA.

restriction enzymes (fragments 1–358 and 343–526) or amplified with the following primers: sense nt 1–20 (5'-GAAGTGGGAGGACAGCGC-3'), nt 2124–2146 (5'-GTGTATGTACAAAAGTCCACAGC-3'), and antisense nt 623–644 (5'-CTGTTGTCCTCCCACTTCTGAGG-3'), nt 819 to 838 (5'-CA-GAATGGGGAGGACAGCCG-3'); antisense nt 623–644 (5'-TTCTT-CCTGCACCCACACACGG-3'), nt 819 to 838 (5'-CAAACTTCTGCTCAGGTCCACAGG-3'). Sense primers contained in addition the T7 RNA polymerase start site. B, antisense deoxyoligonucleotides complementary to portions of the fragment encompassing nt 343–423 (A, nt 343–362; B, nt 361–375; C, nt 376–390; D, nt 391–405; E, nt 406–423) were assayed for their effect on protein-RNA complex formation by preincubating them at a 60-fold molar excess with the labeled TNFR-55 mRNA, followed by addition of extracts from untreated and TPA-treated U-937 cells to protein-RNA complexes formed. C, 32P-labeled fragments of TNFR-55 RNA were generated by RNase H digestion of the labeled RNA fragment containing nt 343–423 after hybridizing it to the appropriate antisense deoxyoligonucleotides (A–D for generation of fragment I; A–C for generation of fragment II; E for generation of fragment III). The fragments were incubated without or with extracts from untreated (Con) and TPA-treated U-937 cells (TPA). Protein-RNA complexes were analyzed as described in the legend to Fig. 3 (asterisk, fragments electrophoresed without prior RNase T1 digestion to control their integrity; IC, intermediate complexes). In the DNA sequence corresponding to fragment II, the 18 nt containing the region essential for formation of complex B and intermediate complexes is in lower case letters.
transcripts (36, 37). The stability of estrogen receptor mRNA was found decreased in response to TPA (68). Furthermore, it has been shown that TPA up-regulates adenosine-uridine binding factor activity in the human T cell line Jurkat (69). The mechanisms underlying those changes are likely to be different from the ones characterized herein. First, the target sequence identified in the TNFR-55 RNA does not show apparent homology in primary structure to sequences involved in the response to TPA in other transcripts (35–37). Second, the time courses of the changes in post-transcriptional mechanisms cited above are rapid, compared that of the TPA-induced changes in TNFR-55 mRNA expression and in the pattern of complexes it forms with proteins. The latter occur only within many hours and thus are more likely to be an indirect consequence of TPA treatment, associated with the differentiation into macrophage-like cells.

For cells of the monocyte/macrophage lineage, which are the main producers of TNF, down-modulation of the TNFR-55 receptors may be especially crucial, limiting an autocrine stimulation of those cells. Of note, transient down-modulation of monocyte cell surface TNFR-55 has been observed in response to lipopolysaccharide (70). The decrease in mRNA in the TPA-treated myelomonocytic cell lines is partial and thus appears to be one of several mechanisms that lead to the complete loss of TNFR-55 from the surface of those cells (19) and unpublished observations.3 To which extent decreased translation, increased degradation, or increased shedding of the TNFR-55 contribute to this phenomenon is not known at present.

TNF induces most of its known cellular effects, through which it takes part in the regulation of inflammatory and immunological responses, by triggering the TNFR-55. Responses elicited by this receptor species can be extremely harmful to the organism. Its damaging effects are also apparent on the immunological responses, by triggering the TNFR-55, which it takes part in the regulation of inflammatory and phago-like cells.

Acknowledgments—We thank Shulamit Michaeli, Oliver Kemper, and David Wallach for helpful discussions.

REFERENCES
1. Tracey, K. J., and Cerami, A. (1993) Annu. Rev. Cell Biol. 9, 317–343
2. Loetscher, H., Pan, Y.-C. E., Lahn, H.-W., Gentz, R., Brockhaus, M., Tabuchi, H., and Lesslauer, W. (1990) Cell 61, 351–359
3. Schall, T. J., Lewis, M., Koller, K. J., Lee, A., Rice, G. C., Wong, G. H. W., Althage, A., Zinkernagel, R., Ohashi, P. S., Konrak, M., and Mak, T. W. (1993) Cell 73, 457–467
4. Nophar, Y., Kemper, O., Brakebusch, C., Engelmann, H., Zwang, R., Aderka, D., Holtmann, H., and Wallach, D. (1990) Cell 63, 361–370
5. Chen, F. Y., Amara, F. M., and Wright, J. A. (1994) J. Biol. Chem. 269, 6709–6715
6. Rothe, J., Blüthmann, H., Gentz, R., Lesslauer, W., and Steinmetz, M. (1993) J. Exp. Med. 177, 1741–1745
7. Winzen, R., Wallach, D., Engelmann, H., Nophar, Y., Brakebusch, C., Kemper, O., Resch, K., and Holtmann, H. (1992) J. Biol. Chem. 267, 3434–3460
8. Schall, T. J., Lewis, M., Koller, K. J., Lee, A., Rice, G. C., Wong, G. H. W., Althage, A., Zinkernagel, R., Ohashi, P. S., Konrak, M., and Mak, T. W. (1993) Cell 73, 457–467