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Analysis of p60 and p80 Tumor Necrosis Factor-α Receptor Messenger RNA and Protein in Human Placentas

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Tumor necrosis factor-α (TNF), a pleiotrophic, multifunctional polypeptide factor, has been reported in both normal and infected human placentas. To identify potential targets for this cytokine, the cells in early and late gestation placentas and extraplacental membranes that express the two TNF receptor (TNF-R) genes, p60 and p80, were identified by using* in situ *hybridization and immunocytochemistry. Gestation-related, cell lineage-specific differences in steady-state levels of p60 and p80 TNF-R messenger RNA were observed. p60 TNF-R messenger RNA predominated at both early and late stages of gestation, being high in both mesenchymal and trophoblastic cell lineages. By contrast, p80 TNF-R messenger RNA was abundant only in intermittent stretches of first trimester syncytiotrophoblast and term placental mesenchymal cells. Overall, intensities of the TNF-R hybridization signals were stronger in term than in first trimester tissues. Transcription of the two TNF-R genes was confirmed by Northern blot hybridization. Translation was verified in all samples by immunohistology using polyclonal antibodies specific for the receptor proteins. p60 and p80 TNF-R proteins were identified both intracellularly and in maternal and fetal blood. Because TNF-Rs exist in both membrane-bound and soluble forms, the results of this study are consistent with the postulate that placental TNF-R have two critical functions: 1) modulation of TNF utilization by specific placental cell lineages during the course of pregnancy; and 2) protection against excessive TNF produced during infections. (Am J Pathol 1993, 143:1131–1141)

Tumor necrosis factor-α (TNF), a polypeptide factor first associated with macrophage activation, wasting in tumor-bearing animals and septic shock,[1–4] is now known to be produced by many types of cells in the absence of infection and inflammation.[5–8] TNF has pleiotrophic effects on proliferation,[9–11] modulates cellular differentiation,[12,13] influences hormone secretion,[14] and exerts a host of other effects on cellular functions.[2–4] Consequently, endogenous TNF has been postulated to serve as a modulator of normal tissue homeostasis.[8,15] Multiple regulatory mechanisms control TNF transcription, message stability, translation, and ultimate secretion of bioactive protein.[4,16] Soluble TNF receptors (TNF-Rs) have been identified in urine and are believed to play a major role in regulating levels of bioactive TNF.[17]

Mapping studies have shown that the TNF gene is transcribed and translated in mouse ovaries,[18] cycling human endometria, [19] and rat, mouse, and human decidua and placentas.[20–23] Although the functions of this potent cytokine are largely unknown, TNF stimulates placental hormone secretion,[14] exogenously administered TNF restores fertility in a mouse model of spontaneous embryo resorption,[24] and administration of anti-TNF interferes with normal development of mouse embryos.[25] Collectively, therefore, the observations support the postulate that TNF contributes positively to reproductive processes. TNF homeostasis in this organ is clearly of major importance; high levels of TNF are associated with pathological consequences that include preterm labor, placental hemorrhage, and embryonic death.[26,27] Protection from the detrimental effects of excessive TNF might be provided by soluble TNF-Rs, which are found in the amniotic fluid and urine of pregnant women.[28]

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TNF-Rs, termed p60 (or p55) and p80 (or p75) to reflect their molecular masses, seem to be expressed on the membranes of nearly all cells albeit in different proportions.[29–32] The two proteins have similar extracellular TNF binding domains but dissimilar cytoplasmic domains, and their affinities for ligand are also different. Several intracellular signaling pathways are activated by ligand-receptor interactions, and receptor species as well as cell lineage and cytokine concentration influence the ultimate outcome.[33–37] Soluble forms of the TNF-Rs compete with membrane receptors, interfering with ligand binding and signal transduction. Thus far, only term placentas have been evaluated for TNF-Rs. Cell lysates contain TNF binding proteins,[38] and messenger RNA (mRNA) for both p60 and p80 TNF-Rs have been identified by Northern blot hybridization.[29,30]

Identification of the cellular sources of the TNF binding proteins in human placentas would permit predictions regarding utilization of this potent cytokine under normal conditions of pregnancy as well as the potential of the placenta to resist damage by excessive TNF. We therefore analyzed first trimester placentas, term placentas, and term extraplacental membranes for TNF-R mRNA by *in situ* hybridization procedures, confirming our results on first trimester tissues by Northern blot hybridization. Immunohistology was performed on semiserial sections of placentas to identify the translated products of messages from the two TNF-R genes.

Materials and Methods

Tissues

First trimester placentas (n = 3) were obtained from elective pregnancy terminations, and term placentas (n = 3) were obtained from cesarean sections performed to avoid fetal distress. The tissues were acquired in cooperation with the Department of Obstetrics and Gynecology under a protocol approved by the Human Subjects Committee of this institution. First trimester placenta was separated by manual dissection from other components. Samples of term placentas were taken between the basal and chorioallantoic plates. Membrane samples were taken from reflected amniochorion (chorion laeve and amnion) opposite to the chorioallantoic plate. Portions of first trimester placentas were flash-frozen in liquid N_2 for later harvesting of RNA. 0.5 to 1.0 cm^3 samples from all of the tissues were fixed immediately in freshly prepared buffered 4% paraformaldehyde for paraffin embedding and *in situ* hybridization as described previously.[20,22]

Molecular Probes

A 1-kb *Eco*RI insert of cDNA specific for the p60 TNF-R cloned into a Gemble-based plasmid and a 640-bp *Not*I/*Bgl*II fragment specific for the p80 TNF-R cloned into the Blueskript SK vector (Stratagene, La Jolla, CA)[29,31] were gifts from C. Smith, Immunex Corp., Seattle, WA. The inserts were verified for size on agarose gels after treatment with appropriate restriction enzymes.

Northern Blot Hybridization

Samples taken from first trimester placentas and pellets of the human myelomonocytic cell line U937 (CRL1593, American Type Culture Collection, Rockville, MD) were homogenized. The U937 cells were grown in tissue culture flasks in RPMI 1640 containing 10% FBS, antibiotics and glutamine. Total cell RNA was extracted by using the guanidium isothiocyanate method,[39] and poly(A)$^+$RNA was prepared using described methods.[40] Equal amounts of the preparations (~2.5 μg) were subjected to electrophoresis on agarose gels. RNA was blotted onto nylon membranes and the blots were hybridized with ^{32}P-labeled antisense p60 and p80 TNF-R RNA probes (2×10^6 cpm/ml) using standard methods.[41]

In Situ Hybridization

The *in situ* hybridization procedure was based on that of Lawrence and Singer[42] with modifications that have been reported.[22,43] Plasmids were linearized using restriction enzymes (antisense p60 TNF-R, *Bam*HI; sense p60, *Pvu*II; antisense p80, *Not*I; sense p80, *Eco*RI). Biotinylated antisense and sense RNA probes were transcribed using Biotin 11-UTP (Enzo Diagnostics, Syosset, NY), and polymerases (antisense p60 and p80 TNF-R, T7; sense p60, SP6; sense p80, T3) from Promega Biotec, Madison, WI. Cocktails prepared as described earlier[22,43] that contained equal concentrations of the biotinylated antisense and sense probes were hybridized with tissue sections pretreated with 1 μg/ml of proteinase K (Sigma Chemical Co., St. Louis, MO) and acetic anhydride.[43] To block nonspecific binding of the sense p60 TNF-R to acellular elements of the tissues, 2 μg/ml of unlabeled vector RNA was included in the hybridization solutions containing the labeled probes (Enzo Diagnostics

Technical Bulletin). After high stringency washes, hybridization was detected using streptavidin-alkaline phosphatase and a nitroblue tetrazolium/bromo-chloroindolyl phosphate-containing substratefor the enzyme (Promega Biotec) that gave a deep blue-brown positive hybridization signal. Hybridization with the p60 TNF-R antisense RNA probe was maximal after 1.5 hours of incubation in substrate, whereas hybridizations with the p80 TNF-R required 6 to 8 hours of incubation. Samples of first trimester placenta, term placenta and amniochorion were tested in the same experiment to permit comparisons of the hybridization signals. The tissues were counterstained with methyl green. Other controls consisted of pretreating some tissue sections with RNase A and substituting transfer RNA for the specific probes as described before[22,43] so as to identify any nonspecific hybridization signals.

Immunocytochemistry

Tissue sections from the same blocks of first trimester and term placentas that had been used for the *in situ* hybridization experiments were tested by immunocytochemistry with rabbit antibodies to the p60 TNF-R (1:50 dilution recommended for immunocytochemistry, Cytokine Sciences, Boston, MA) and to the p80 TNF-R (P5, 1:100 dilution, a gift from C. Smith, Immunex). These antibodies have been extensively characterized for specificity by the suppliers. Normal rabbit serum (1:50 or 1:100, Sigma) was used to identify nonspecific binding in semiserial sections of the tissues. The experiments were done as described previously,[22] with binding detected using an anti-rabbit IgG kit from Zymed (South San Francisco, CA). The tissue sections were lightly counterstained with Gill's hematoxylin.

Results

Distribution of p60 TNF-R mRNA in Placentas and Membranes

Figure 1 shows that p60 TNF-R mRNA was readily detected in first trimester placenta, term placenta, and term extraplacental membranes by using *in situ* hybridization procedures. In first trimester placentas (Figure 1, A and B), stromal cells as well as endothelial cells lining stem vessels in essentially all villi contained p60 TNF-R message, and signals were invariably strong. By contrast, hybridization signals were extremely variable in villous syncytiotrophoblast and cytotrophoblast cells. While trophoblast in approximately 90% of the villi contained p60 TNF-R mRNA, within a single villous, areas of strong, weak, and no signal were common (Figure 1A). In an estimated 40% of villi, signals in syncytiotrophoblast predominated, whereas in approximately 30% of the villi, syncytiotrophoblast was negative and the underlying cytotrophoblast cells were strongly positive. These distinct patterns are shown in Figure 2A. Cells in cytotrophoblastic columns emerging from the villi (extravillous cytotrophoblast cells) consistently exhibited high levels of p60 TNF-R message (Figure 2B).

In term placentas (Figure 1, D and E), hybridization signals remained consistently strong in mesenchymal and endothelial cells. Differences between mesenchymal cell and syncytiotrophoblast p60 TNF-R mRNA were less striking than in first trimester placentas. Most syncytiotrophoblast contained p60 TNF-R message, although signal intensity in this cell layer was generally lower than in the mesenchymal cells. Overall, term placentas appeared to contain somewhat higher levels of p60 TNF-R mRNA than first trimester placentas because of greater uniformity in syncytiotrophoblast. In term membranes (Figure 1G), signal intensity was slightly higher in decidua than in adjacent chorion membrane cytotrophoblast cells. Accumulations of transcripts were observed in some cytotrophoblast cell nuclei (Figure 1G).

The hybridization patterns reported above were consistent among samples of each type that were tested. The sense version of the probe did not hybridize to tissue sections, as shown in Figure 1, C, F, and H. Other types of controls where tRNA was substituted for the specific probes and tissue sections were pretreated with RNase A were negative.

Distribution of p80 TNF-R mRNA in Placentas and Membranes

Figure 3 shows that p80 TNF-R mRNA was also present in all samples that were tested. In first trimester placentas (Figure 3A), an estimated 70% of all villi failed to demonstrate any p80 TNF-R transcripts. In villi containing p80 TNF-R message, the gene was primarily expressed in intermittent stretches of syncytiotrophoblast (Figure 3A). In contrast to p60, p80 TNF-R signals were low in mesenchymal cells (Figure 3A) as well as in villous and extravillous (Figure 3B) cytotrophoblast cells.

In term placentas, essentially all villi contained mesenchymal cells that were strongly positive for

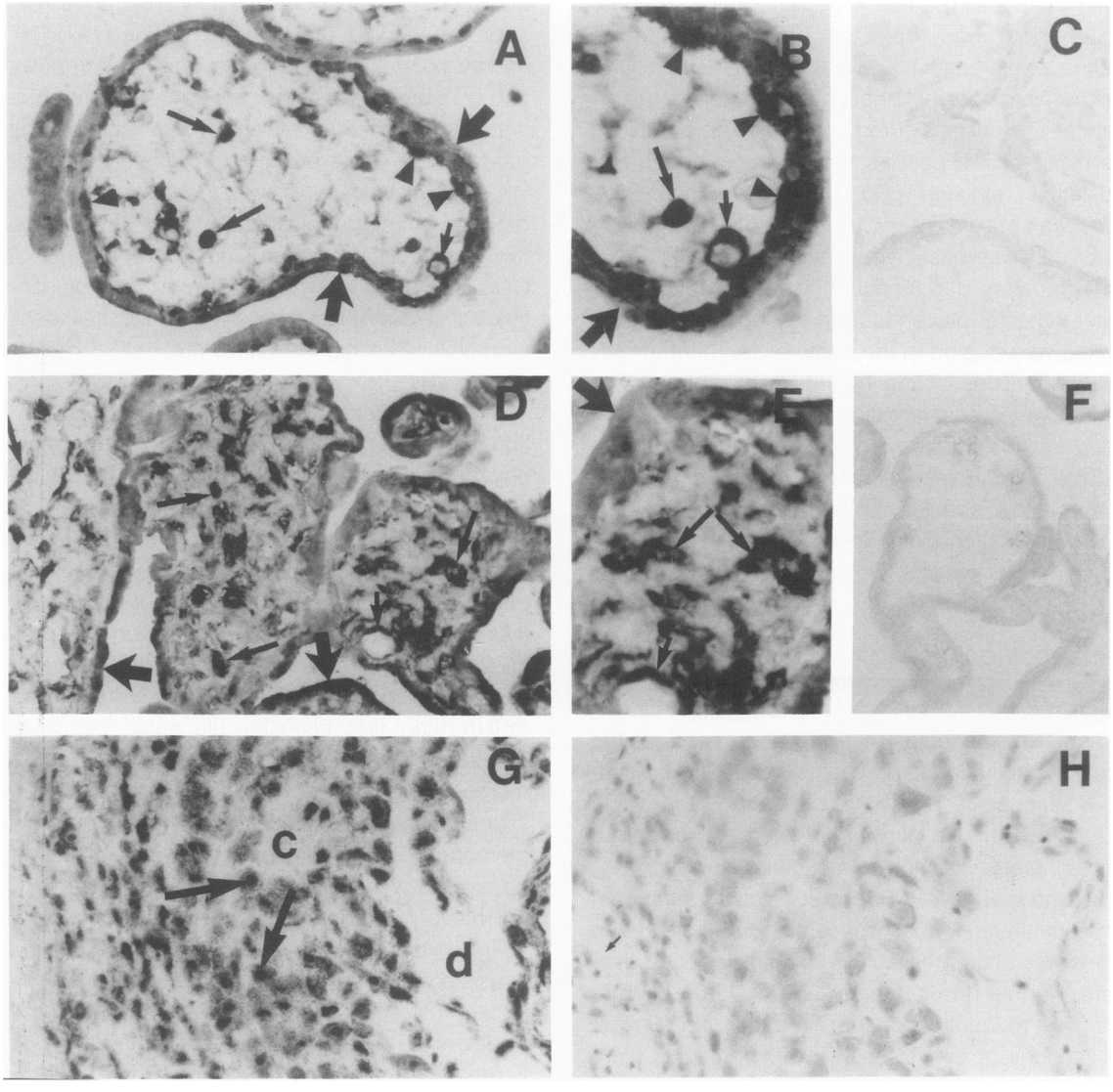

Figure 1. *Identification of p60 TNF-R mRNA in first trimester placenta, term placenta, and term extraplacental membranes by in situ hybridization.* **A, B, D, E** *and* **G** *show hybridization patterns with the p60 TNF-R antisense probe.* **C, F,** *and* **H** *show that signals were absent when the sense version of the p60 TNF-R probe was hybridized with semiserial sections of the same tissues.* **A:** *First trimester placenta. Strong hybridization signals were present in stromal cells* (**long arrows**) *and endothelial cells* (**short arrow**). *Both cytotrophoblast cells* (**arrowheads**) *and syncytiotrophoblast* (**large arrows**) *in some areas contained specific message.* **B:** *Enlargement (×2) of a portion of the same photomicrograph. Arrows point out the same subpopulations as in* (**A**). **D:** *Term placenta. p60 TNF-R transcripts were present in stromal cells* (**long arrows**), *endothelial cells* (**short arrow**), *and syncytiotrophoblast* (**large arrows**). **E:** *Enlargement (×2) of a portion of the same photomicrograph. Arrows point out the same subpopulations as in* (**D**). **G:** *Term extraplacental membranes. The p60 TNF-R antisense RNA probe identified message in chorion membrane cytotrophoblast cells* (**c**), *and concentrations of specific message in their nuclei* (**arrows**). *Decidual cells* (**d**) *contained slightly higher levels of message than cytotrophoblast cells. Original magnifications ×250.*

p80 TNF-R mRNA (Figure 3, D and E). While the majority of syncytiotrophoblast contained detectable p80 TNF-R message, hybridization signals in this cell layer were strikingly less intense than in the mesenchymal cells. Overall, term placentas appeared to contain higher levels of p80 TNF-R mRNA than first trimester placentas. In contrast to p60, this was due primarily to stronger signals in mesenchymal cells. In the extraplacental membranes (Figure 3G), decidua exhibited stronger hybridization signals than the adjacent chorion membrane cytotrophoblast cells.

The hybridization patterns reported above were consistent among samples of each type that were tested. Controls that consisted of hybridizing each tissue with the sense orientation of the p80 TNF probe (Figure 3, C, F, and H), as well as other types of controls where tRNA was substituted for the spe-

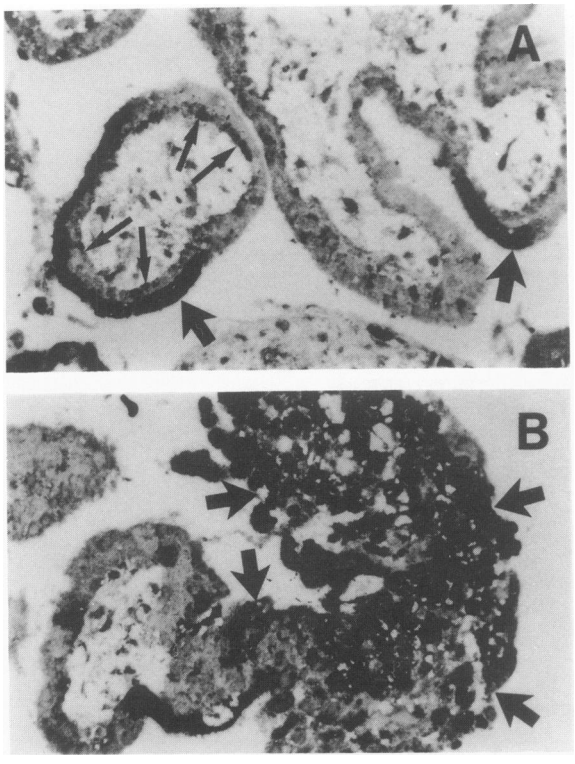

Figure 2. *p60 TNF-R transcripts in subpopulations of first trimester trophoblast.* **A:** *A villous in which message was concentrated in discrete stretches of syncytiotrophoblast* (**large arrows**), *and was present in cytotrophoblastic cells* (**small arrows**). **B:** *Cells in a cytotrophoblastic column* (**large arrows**) *emerging from a placental villus exhibited intense hybridization signals. Original magnifications ×250.*

cific probes and tissue sections were pretreated with RNase A, were negative.

Identification of TNF-R mRNA by Northern Blot Hybridization

Because TNF-R mRNA has not been previously reported in first trimester tissues, transcription was confirmed by using Northern blot hybridization procedures. Figure 4 shows that poly(A)$^+$ RNA preparations from first trimester placenta contained both p60 and p80 TNF-R mRNA. p60 mRNA in lysates of first trimester placenta migrated to the same position on the agarose gels, 2.3 kb, as has been reported for this receptor species in term placentas.[30] Consistent with a previous report,[31] the U937 cell preparations tested in these experiments contained 2.3-kb p60 TNF-R transcripts. No additional bands were detected by extended exposure of the autoradiograms.

Northern blot analysis revealed two p80 TNF-R mRNA species in poly(A)$^+$ RNA preparations from first trimester placenta and U937 cells that migrated to 5.0 and 4.5 kb positions on the gels (Figure 4). The 5.0 kb species predominated in both preparations. While the minor 4.5-kb mRNA in placenta poly(A)$^+$ RNA was readily identified, detection of this receptor species in the U937 cells required longer exposure of the autoradiograms (shown in Figure 4). Term placenta has been reported to contain the 4.5-kb TNF-R transcript,[29] whereas transcript(s) in U937 cells have not been reported.

Immunocytochemical Identification of TNF-R Protein in Placentas

The same first trimester and term placenta tissue blocks examined by *in situ* hybridization for p60 and p80 TNF-R mRNA were tested by immunocytochemistry using polyclonal antibodies to p60 and p80 TNF-R proteins.

All samples contained both p60 and p80 TNF-R protein. Figure 5, A and B, show that while mesenchymal cells in first trimester villi contained little p60 TNF-R protein, trophoblastic cells were positive, with staining being strongest in syncytiotrophoblast. p60 TNF-R protein was also present in maternal blood. In term placentas (Figure 5, D, and E), where cytotrophoblastic cells are scarce, syncytiotrophoblast remained the major cellular locale of p60 TNF-R protein. Immunoreactive protein was homogeneously distributed within cell cytoplasm rather than concentrated as described below for p80 TNF-R protein. p60 TNF-R protein was frequently present in placental stem vessels (Figure 5, D and E). These results were consistent in the three samples from each gestational stage that were tested. Normal rabbit serum did not bind to the tissue sections (Figure 5, C and F).

As shown in Figure 6, A and B, mesenchymal cells in first trimester villi contained little specific p80 TNF-R protein. Immunoreactive protein was present in intermittent stretches of syncytiotrophoblast and in cytotrophoblast cells. Both cellular and acellular elements of maternal blood demonstrated reactivity with anti-p80 TNF-R. In term placentas (Figure 6, D and E), approximately 10% of the mesenchymal cells were strongly positive with the specific antibody, while syncytiotrophoblast contained only low levels of p80 TNF-R protein. In contrast to the homogeneous staining with the anti-p60 antibody described above, staining with anti-p80 was granular in both lineages, suggesting discrete localization within the cytoplasm. In one of three term placentas, p80 TNF-R protein was prominent in pla-

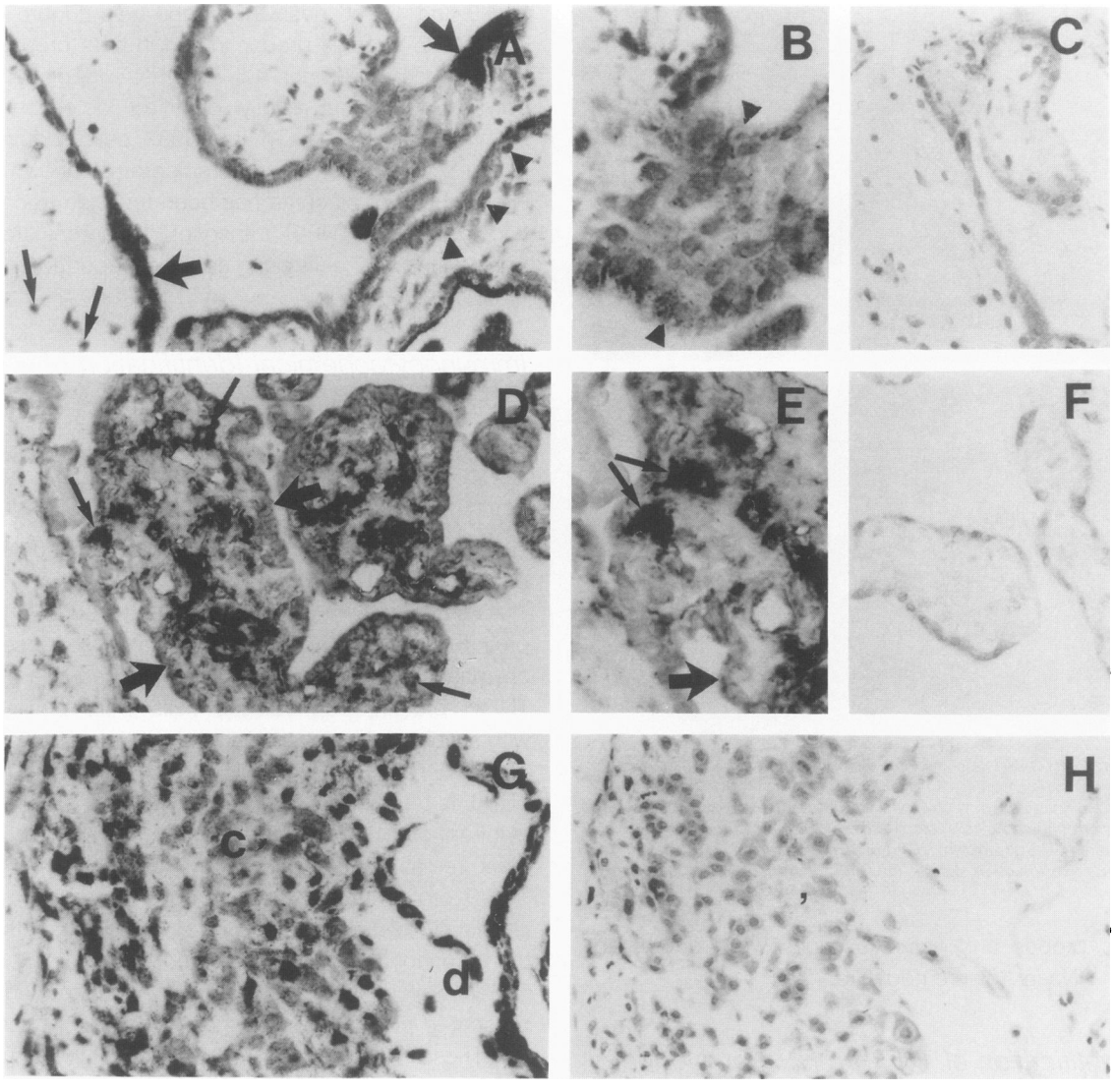

Figure 3. *Identification of p80 TNF-R mRNA in first trimester placenta, term placenta, and term extraplacental membranes by* in situ *hybridization.* **A, B, D, E,** *and* **G** *show hybridization patterns with the antisense probe.* **C, F,** *and* **H** *show that signals were absent when the sense version of the p80 TNF-R probe was hybridized with semiserial sections of the same tissues.* **A:** *First trimester placenta. Intermittent stretches of syncytiotrophoblast* (**large arrows**) *exhibited strong hybridization signals, villous cytotrophoblastic cells* (**arrowheads**) *were usually negative and some mesenchymal cells* (**small arrows**) *were weakly positive.* **B:** *Enlargement (×2) of a portion of the same photomicrograph showing that hybridization signals were weak in cells contained in a cytotrophoblastic column* (**arrowheads**). **D:** *Term placenta. p80 TNF-R transcripts were present in villous stromal cells* (**small arrows**), *whereas syncytiotrophoblast* (**large arrows**) *contained less specific message.* **E:** *Enlargement (×2) of the same photomicrograph* (**large arrow**, *syncytiotrophoblast;* **small arrows**, *mesenchymal cells*). **G:** *Term extraplacental membranes. Chorionic cytotrophoblast cells* (**c**) *contained less p80 TNF-R message than decidual cells* (**d**). *Original magnifications ×250.*

cental stem vessels (not shown). Normal rabbit serum did not bind to the tissue sections (Figure 6, C and F).

Summary

Table 1 summarizes the results obtained when human placentas were tested for mRNA and proteins derived from the two TNF-R genes. Of particular note, steady state levels of p60 TNF-R mRNA were stable through gestation in specific placental cell lineages in comparison with p80 TNF-R mRNA. TNF-R proteins in mesenchymal cells were less prominent than would be expected from the *in situ* hybridization results. Immunoreactive TNF-R proteins were present in maternal blood in first trimester tissues and in fetal blood vessels in term placentas.

Discussion

The results of this study were in accord with previous observations on TNF-R gene expression in human placentas. Consistent with reports of TNF-R

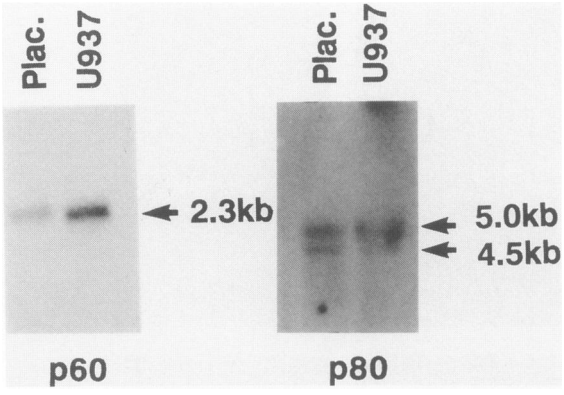

Figure 4. *Northern blot hybridization analysis of p60 and p80 TNF-R mRNA in poly(A)$^+$ RNA preparations from first trimester placentas and the U937 human myelomonocytic cell line. Placenta and U937 preparations contained a single species of p60 TNF-R mRNA, which migrated to the same position, 2.3 kb, on the gel. Two species of p80 TNF-R mRNA (5.0 and 4.5 kb) were present in placental and U937 poly(A)$^+$ RNA. Equal cpm of ^{32}P-labeled p60 and p80 TNF-R RNA probes were used for the hybridizations (2×10^6 cpm/ml), and equal amounts of poly(A)$^+$ RNA (~2.5 μg) were loaded into each lane. The p60 blot was exposed to x-ray film for 48 hours and the p80 blot was exposed for 10 days.*

mRNA in term placentas by Northern blot hybridization[29,30] and immunoreactive TNF-R in frozen sections of term placentas,[44] we observed both p60 and p80 TNF-R mRNA in late gestation placentas by using *in situ* hybridization and protein in the same paraformaldehyde-fixed tissues. Our results showing TNF-R mRNA and protein in early gestation placentas were in accordance with a recent report of p60 and p80 TNF-binding proteins in the urine and amniotic fluid of pregnant women.[28] Subjective evaluations of tissues tested by *in situ* hybridization and immunocytochemistry indicated that p60 and p80 TNF-R message and protein were higher at term than in first trimester tissues, which is consistent with the observation that binding proteins are more abundant in term than in first trimester fluids.[28]

The use of *in situ* hybridization as an experimental approach permitted us to learn that steady-state levels of p60 TNF-R mRNA are consistently high in the two embryologically distinct lineages of placental cells, mesenchymal cells and trophoblast, during the course of pregnancy. By contrast, levels of p80 TNF-R mRNA in both lineages are dependent on stage of gestation. In mesenchymal cells, high intensity p80 TNF-R signals were present only in term tissues. Many of these cells are macrophages, which gradually acquire MHC class II antigens and other maturation and activation-associated markers during gestation.[45–47] In the trophoblast lineage, which differentiates into phenotypically and functionally distinct subpopulations during the course of pregnancy,[48] high intensity p80 hybridization signals were restricted to syncytiotrophoblast in first trimester tissues. In other contexts, TNF influences cellular differentiation, maturation and activation via binding to specific receptor species.[12,13,34–36,49] Thus, it does not seem unreasonable to propose causal associations between differential expression of membrane-bound TNF-R proteins, receptivity to endogenous TNF, and placental cell maturation and development. These could include autocrine, juxtacrine, and paracrine pathways similar to those indicated in studies on other uteroplacental growth factors.[50–52]

Potential effects of TNF binding to placental cell TNF-R include but are not limited to cell lineage-specific modulation of growth and MHC class I expression,[9–11] stimulation of angiogenesis,[53] regulation of synthesis of enzymes such as collagenase[54] required for tissue remodeling, protection against viral infections,[45] and inhibition of lymphocyte proliferation.[55] All would be useful activities in expanding semi-allogeneic placentas and embryos. The observation that first trimester placental lysates contained both 4.5- and 5.0-kb p80 TNF-R mRNA, while term placental lysates have been reported to contain only the smaller transcript,[29] was of considerable interest. Whether or not this might ultimately be reflected in functionally distinct proteins important to specific stages of gestation remains to be seen.

Soluble receptors might perform equally important tasks, protecting placentas and embryos against excessive endogenous TNF[22] as well as extremely high levels produced during infections.[27] Our immunohistochemical observations confirmed translation of the TNF-R mRNAs into protein, and showed that both maternal and fetal blood contained soluble receptors. Regarding the cellular origin of these receptors, syncytiotrophoblast, which contained both TNF-R mRNA and protein, was a less likely source than mesenchymal cells. Despite the presence of TNF-R mRNA, neither p60 and p80 TNF-R protein was particularly abundant in mesenchymal cells (Table 1), a finding that has also been reported by Austgulen et al,[44] who tested frozen sections of term placentas using monoclonal reagents. One interpretation of these observations is that the mesenchymal cells export much of their protein, a postulate supported by the identification of receptor proteins in maternal blood in first trimester tissues and fetal stem vessels at term. Alternatively, TNF-R message in these cells may have been stored and not translated. Although this has been

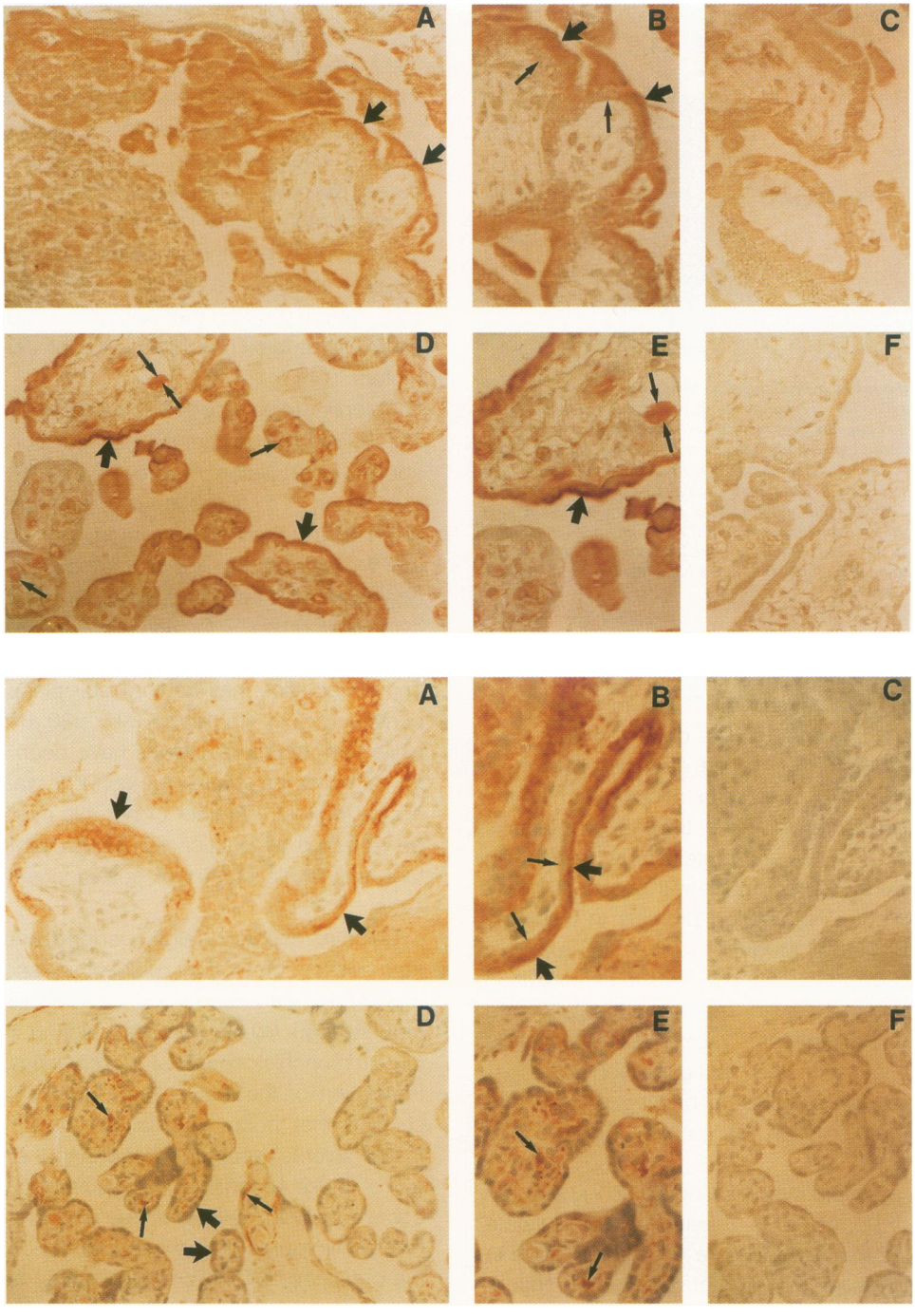

Figure 5. *Identification of p60 TNF-R protein in first trimester and term placentas by immunohistochemistry.* A, B, D, *and* E *show binding of rabbit anti-human p60 TNF-R to placental cells.* C *and* F *show that normal rabbit serum did not bind to the tissue sections.* A: *In first trimester placental villi, immunoreactive p60 TNF-R protein was localized primarily to syncytiotrophoblast* (large arrows). B: *Enlargement* (×2) *of the same photomicrograph illustrates antibody binding to syncytiotrophoblast* (large arrows). *Villous cytotrophoblastic cells* (small arrows) *and mesenchymal cells in the centers of placental villi contained little p60 protein.* D: *In term placenta, mesenchymal cells contained little p60 protein, while syncytiotrophoblast* (large arrows) *was positive, and protein was present in placental stem vessels* (small arrows). E: *Enlargement* (×2) *of the same photomicrograph showing immunoreactive protein in syncytiotrophoblast* (large arrow) *and a placental blood vessel* (arrowhead). *Original magnifications* ×200.

Figure 6. *Identification of p80 TNF-R protein in first trimester and term placentas by immunohistochemistry.* A, B, D *and* E *show binding of rabbit anti-human p80 TNF-R to placental cells.* C *and* F *show that normal rabbit serum did not bind to the tissue sections.* A: *In first trimester placenta, mesenchymal cells were negative, while p80 TNF-R protein was concentrated in intermittent stretches of trophoblastic cells* (large arrows). B: *Enlargement* (×2) *of the same photomicrograph illustrating antibody binding to syncytiotrophoblast* (large arrows). *Villous cytotrophoblastic cells* (small arrows) *contained less p80 TNF-R protein.* D: *In term placenta, p80 TNF-R protein was prominent in some mesenchymal cells* (small arrows) *but was low in syncytiotrophoblast* (large arrows). E: *Enlargement* (×2) *of the same photomicrograph showing immunoreactive mesenchymal cells* (small arrows). *Original magnifications* ×200.

Table 1. *Summary of TNF-R mRNA and Protein in Human Placentas**

	p60 TNF-R		p80 TNF-R	
	mRNA	Protein	mRNA	Protein
First trimester placentas				
Syncytiotrophoblast	++ (I)	++ (I)	++ (I)	++ (I)
Villous cytotrophoblasts	++ (I)	+ (I)	± (I)	++ (I)
Stromal cells	++	0	± (I)	± (I)
Maternal blood	ND†	++	ND	++
Fetal blood	ND	0	ND	0
Term placentas				
Syncytiotrophoblast	++	++ (I)	±	± (I)
Stromal cells	++	±	++	++ (I)
Fetal blood	ND	++	ND	+ (I)

* Hybridization signal intensities and immunostains were estimated as 0 = negative; ± = weak positive; + = positive; ++ = strongly positive. I indicates inconsistencies in hybridization signals or antibody binding.

† ND = not determined due to an insufficiency of appropriate cells or blood in the samples.

shown for the complementary factor, TNF,[56] message accumulation has not been reported for TNF-R mRNA.

In conclusion, the results of these experiments permit new insights into two critical potential roles for TNF-R in human placentas, modulation of normal growth and differentiation and protection against the dangerous effects of high levels of TNF. Our experimental results are therefore consistent with the postulate that TNF-R expression in placentas will have a major impact on the success of both normal and complicated pregnancies.

Acknowledgments

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