Int. J. Dev. Biol. 33: 173-176 (1989)

Tumor necrosis factor in the human fetoplacentary unit

EERO SAKSELA and MARJA JÄÄTTELÄ

Department of Pathology, University of Helsinki, Haartmaninkatu 3, SF-00290 Helsinki, Finland

ABSTRACT. In the present paper we review our findings on tumor necrosis factor (TNF) in human placental and adrenal tissues and suggest a possible novel biological role for this multi-functional lymphokine. TNF was found in the second and third trimester amniotic fluids in concentrations varying from 1.7 to 2.5 ng/ml on the average. In the amniotic fluid TNF was present in a biologically inactive form but in freshly-prepared supernatants of placental and decidual tissue homogenates corresponding levels were also found with bioassays based on the cytotoxicity of TNF to WEHI cells. In screening other fetal tissues we could detect biologically active TNF also in homogenates of fetal adrenals, and cultured fetal adrenal cells produced TNF upon stimulation with ACTH. A possible biological function for TNF was suggested by the findings that it suppressed the ACTH stimulated cortisol synthesis of fetal adrenal cultures and switched the steroidogenesis towards dehydroepiandrosterone (DHEA) and its sulphate (DHEAS). These are important precursors for placental steroid synthesis which are mainly provided by the fetal adrenals during normal gestation, and the results thus suggest a role for TNF in the regulation of steroidogenesis in the human fetoplacentary unit.

KEY WORDS: *Tumor necrosis factor, steroid hormones, fetoplacentary unit*

Introduction

Tumor necrosis factor (TNF) was originally described as a bacterial endotoxin-induced agent in mouse blood that caused hemorrhagic necrosis in tumors and lysed cancer cells *in vitro* (Carswell *et al.*, 1975). It was shown to be a product of activated macrophages and the molecule was cloned and sequenced in 1984 (Aggarwal *et al.*, 1985). With the availability of pure recombinant TNF our knowledge of its widespread biological effects has rapidly increased.

In the immune system TNF belongs to the family of immunomodulatory cytokines and has complex regulatory effects on T cells, B-cells and polymorphonuclear leukocytes. It can stimulate angiogenesis and modulates endothelial cell adhesive properties. TNF also interacts with various growth factors by inducing PDGF, IL-1, interferon beta-2 and various colony stimulating factors, and promotes cell differentiation in some experimental systems (for review see Beutler and Cerami, 1988). The systemic effects of an endotoxin-induced inflammatory response are mostly mediated by TNF, which acts as an endogenous pyrogen, induces acute-phase proteins and muscle and fat catabolism. The latter effect has been shown to be mediated by the inhibition of adipocyte lipoprotein lipase and in extended exposure leads to cachexia (Cerami *et al.*, 1985).

To this abridged list of TNF effects we have been able to add another dimension showing that TNF can act as a mediator bridging the immune and endocrine systems because of its presence and modulatory action on the steroidogenesis in the human fetoplacentary unit.

Steroid synthesis in human fetoplacentary unit

The first steroid-producing cells, placental trophoblasts, appear early in human development. Although active in the production of estrogens and progesterone, placenta lacks several key enzymes in steroid metabolism and is therefore dependent on precursors, mainly dehydroepiandrosterone (DHEA) and its sulphate (DHEAS), synthesized in the fetal zone of the fetal adrenal cortex (for review see Villee, 1972). To supply the placental need of high levels of these precursors, the secretory pattern in fetal zone differs greatly from that in the adult zone. Fetal zone synthesizes high levels of DHEA and DHEAS and low levels of glucocorticoids and mineralocorticoids, the main products of the adult zone, reflecting the low activity of 3β-hydroxysteroid dehydrogenase (Fig. 1). Products secreted by placenta have been shown to suppress the activity of this enzyme (Voutilainen and Kahri, 1980), but the mechanism of the suppression is not fully understood.

Presence of TNF in human placental and adrenal tissues

Our origin for these studies was the demonstration of TNF in normal human amniotic fluid (Jäättelä *et al.*, 1988). In 74 normal amniotic fluid samples taken for alphafetoprotein screening during second and third trimesters 67 (91%) contained TNF detectable with RIA(over 0.3 ng/ml) with a mean concentration of 1.7 ng/ml. The concentrations during the second trimester were higher with a mean of 2.5 ng/ml, but in all samples the TNF was found to be present in an inactive form not causing cytotoxicity in susceptible target cells. As the recombinant TNF and monoclonal anti-TNF RIA was shown to be highly specific and not cross-reacting with the 38% homologous lymphotoxin (TNF-beta), the TNF in the amniotic fluid was probably degraded into inactive forms or bound to neutralizing carriers.

However, when supernatants of freshly-prepared placental or decidual tissues were analyzed, biologically active cytotoxic TNF could be detected (Table 1). The concentrations measured by RIA ranged from 1.1 to 2.8 ng/ml in full term placentas and comparison of the biological activity with standard curves obtained with rTNF gave similar quantitative estimates (1.0 - 3.0 ng/ml). In the homogenate supernatants from decidual tissues the levels of biologically active TNF were even higher. The average concentration measured by RIA was 6.7 ng/ml and the corresponding value with bioassay was 7.6 ng/ml. No TNF was found either with RIA or the sensitive bioassay in the control supernatants of homogenates prepared from normal endometrial or myometrial tissues.

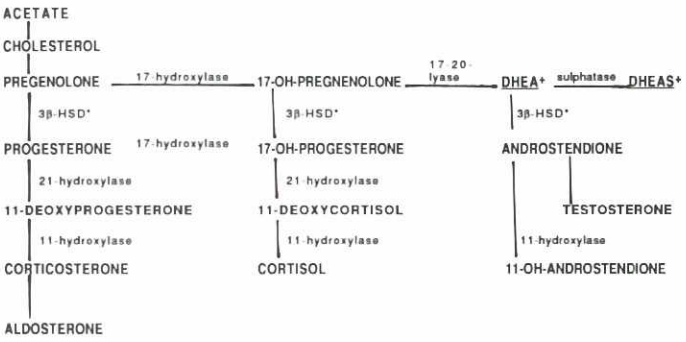

Fig. 1. *Schematic presentation of steroidogenesis in the fetal zone of the fetal adrenal cortex. The main pathway in the fetus is from pregnenolone to DHEAS and the vertical pathways are heavily suppressed in the fetal zone mainly because of the low activity of 3b-hydroxysteroid dehydrogenase.*
* *DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone-sulphate; 3β-HDS, 3β-hydroxysteroid dehydrogenase.*

We then proceeded to study the supernatants of tissue homogenates from several other fetal tissues (Jäättelä *et al.*, in press). The results are shown in Table II. In over half of the cases TNF was detected in the supernatants of adrenal (12/22) and placental (4/7) extracts. TNF was also found in a few of the supernatants of cerebral (2/9), thymic (2/9), pancreatic (1/7), pulmonal (1/9), and hepatic (1/9) extracts but in none of the supernatants of cardiac (9 samples), renal (9), splenic (8), thyroid (7), nor cutaneous (4) extracts. Histologically normal adult adrenals were similarly studied, but none of the 7 samples contained measurable amounts of TNF. When adjusted to the protein concentration of the supernatant the average level of TNF in 11 out of 12 positive adrenal supernatants was 156 pg/mg of protein and the range was 100-210 pg/mg (Table II).

TABLE I

COMPARATIVE HUMAN AMNIOTIC FLUID AND TISSUE SUPERNATANT TNF LEVELS DETECTED BY THE RIA AND THE CYTOTOXICITY ASSAY, AND NEUTRALIZATION OF THE CYTOTOXICITY BY ANTI-TNF

Samples	TNF quantification by		Cytotoxicity	
	RIA	Cytotoxicity assay	Anti-TNF antibody	
			absent	present
	ng/ml		%	
HAF, second trimester, (H15-16)	2.1	0.03	10.4	0.9
HAF, third trimester, (H32-37)	0.9	< 0.03	4.9	1.2
Placental supernatant	1.8	1.9	26.4	0.7
Decidual supernatant	6.7	7.6	34.1	0.2
Normal endometrial supernatant	< 0.3	< 0.03	7.4	4.4
Normal myometrial supernatant	< 0.3	< 0.03	5.1	0.3

Samples were tested in parallel experiments, HAF, = a pool of 5 amniotic fluid samples. Figures for tissue supernatants are average values of 6 full-term placenta, 3 full-term decidua, or 2 normal myometrium and endometrium specimens incubated for 1 day in PBS +0.5% BSA. Coefficient of variation in the RIA is < 5% and in the cytotoxicity assay < 10%.

TABLE II

THE PRESENCE OF TNF IN THE EXTRACTS OF DIFFERENT FETAL TISSUES AND IN ADULT ADRENALS ASSAYED BY THE RIA

Tissue	Positive samples in the RIA	TNF pg/mg protein in positive samples
brain	2/9	135
thyroid gland	0/7	n.a.
lung	1/9	30
heart	0/9	n.a.
thymus	2/9	82
liver	1/9	15
pancreas	1/7	n.d.
spleen	0/8	n.a.
adrenal	12/22	156(±12)*
kidney	0/9	n.a.
skin	0/4	n.a.
placenta	4/7	65(±14)
adult adrenal	0/7	n.a.

* The mean value of 11 adrenal extracts (±SD)
n.a., not applicable
n.d., not determined

We could also detect TNF in supernatants of primary cultures of fetal adrenals which were stimulated with 0.2 mg/ml/day of ACTH. Supernatants of such cultures of fetal adrenals were assayed for TNF by cytotoxicity assay employing WEHI 164 cells as targets. As is shown in Fig. 2, 80% of the supernatants collected after the first week of culture with ACTH (10 samples from 5 different fetuses) contained endogenous lytic activity corresponding to up to 250 pg/ml of rTNF tested in parallel experiments. After the third week of culture 3/6 samples from 3 different adrenals contained lytic activity corresponding to up to 60 pg/ml of rTNF. Addition of monoclonal anti-TNF to the assay neutralized the cytotoxicity.

The effect of TNF on fetal adrenal steroidogenesis

The effect of rTNF on the total synthesis of cortisol was studied in the third week cultures, each well having the same amount of cells. In these cultures the addition of 1.0 ng/ml or 3.0 ng/ml of rTNF every 48 hours suppressed cortisol synthesis by 68.7% (mean of 4 cultures) and 86.7% (mean of 2 cultures) respectively and most of this effect could be neutralized by polyclonal anti-TNF antiserum (Fig. 3). Addition of rTNF (3.0 ng/ml) or rTNF and polyclonal anti-TNF antiserum (240 neutralizing units/ml) to adrenal cultures did not have any effect on cell viability as determined by trypan blue exclusion. Cell viability after the third week of culture was 91.8% (±2.1; ±SD) in control wells, 90,5% (±2.0) in wells with rTNF, and 89.4% (±3.1) in wells with rTNF and its antibody.

To investigate at which stage of steroidogenesis the suppression occurred, the ratios of cortisol concentration to concentrations of other steroids synthesized earlier in the pathway were compared between different treatments. The supernatants of the first and the third week ACTH induced cultures of fetal adrenals with no treatment and those treated for 6 days with 1.0 ng/ml or rTNF, 3.0 ng/ml of rTNF, or 3.0 ng/ml of rTNF with polyclonal anti-TNF every 48 hours of culture were assayed for DHEA, DHEAS, 17-hydroxyprogesterone, androstendione, 11-

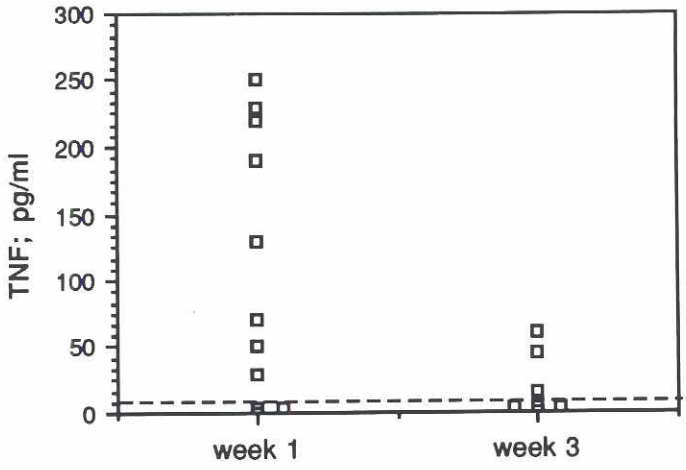

Fig. 2. *Levels of TNF in supernatants of human fetal adrenal cultures detected by a cytotoxicity assay employing WEHI 164 cells as targets. Fetal adrenals were stimulated with ACTH 0.2 μg;ml;day for the first 6 days of culture (week 1) or for days 21-26 of culture (week 3) and supernatants were collected at the end of the stimulation. Detection level marked with a dotted line.*

deoxycortisol, and cortisol by RIAs. In both the first and the third week cultures addition of rTNF significantly lowered the ratio of cortisol production to that of steroids synthesized earlier without affecting significantly the ratio of 11-deoxycortisol to steroids synthesized earlier (Fig. 4). This effect was neutralized by addition of polyclonal anti-TNF to the cultures. Ratios of androstendione concentrations to concentrations of steroids prior in the pathway, e.g. 17-hydroxyprogesterone and DHEA, tended to be higher in cultures treated with rTNF than in cultures with no treatment but the changes were not significant and rTNF had no effect on the ratios of other steroids (data not shown). Because of the cross reaction of 11-deoxycortisol in the cortisol assay (15%), the concentration of cortisol was overestimated by 25-50% in the experiments with TNF. Thus the suppressive effect of TNF on cortisol production was actually larger than that observed.

Comments

High levels of TNF were found in normal amniotic fluids and in incubation supernatants of placental and decidual tissues, and in somewhat lower concentrations in fetal adrenal and placental extracts and in supernatants of fetal adrenals even after 3 weeks of cultivation.

In order to find clues to the possible biological role of TNF in fetal adrenals we investigated the effect of rTNF on the steroidogenesis in primary cultures of human fetal adrenals (Jäättelä *et al.*, submitted). Addition of 1 ng/ml or 3 ng/ml of rTNF into the third week cultures reduced the production of cortisol by 68.7% and 86.7% respectively while not affecting cell viability. The data suggested that addition of rTNF to fetal adrenal cultures decreases the production of cortisol probably by selectively inhibiting the conversion of 11-deoxycortisol to cortisol mediated by 11-hydroxylase while not inhibiting the steps mediated by 3 β-HSD, 21-hydroxylase, 17-20 lyase, or DHEA sulphatase. The effect is dose dependent at least with the two different concentrations of rTNF tested, and it can be neutralized by addition of polyclonal anti-TNF antiserum. In adipocytes TNF has been shown to suppress the activities of a number of different enzymes (Pekala *et al.*, 1983), and Cornelius *et al.*, (1988) have suggested that the suppression occurs at the transcriptional level. TNF has also been shown to regulate oncogene expression by affecting the levels of mRNA (Krönke

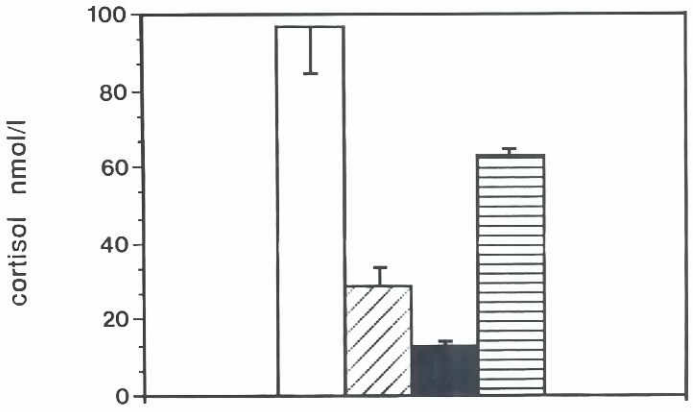

Fig. 3. *Effect of rTNF on ACTH-induced cortisol production in the third week cultures of human fetal adrenals. Values are means (+;− SD) of 4 cultures with no treatment (white bars), 4 cultures with 1 ng;ml of rTNF added every 48 hours of culture (hatched bars, p=0.01), 2 cultures with 3 ng;ml of rTNF added every 48 hours of culture (black bars, p=0.003), and 2 cultures with 3 ng;ml of rTNf and 2 μl;ml of polyclonal anti-TNF antiserum added every 48 hours of culture (cross-hatched bars).*

et al. , 1987; Seliger *et al.*, 1988). One may thus speculate that the suppressed activity of 11-hydroxylase is due to lowered levels of 11-hydroxylase mRNA and we are currently directly testing this hypothesis.

Basically these results provide evidence of a novel interaction between the endocrine and immune systems which could

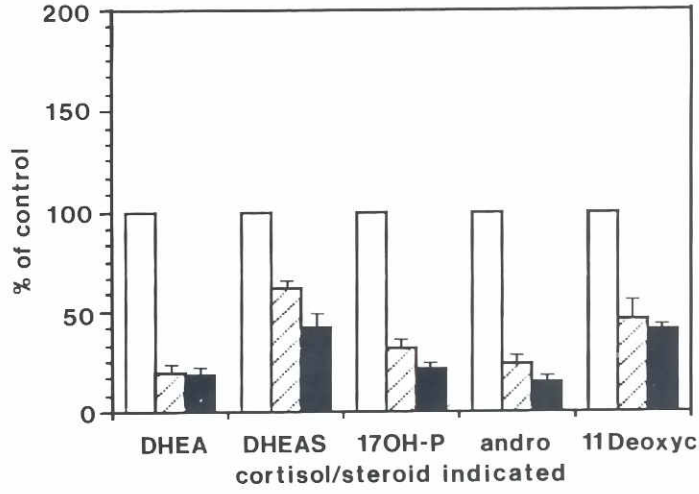

Fig. 4. *Effect of rTNF (1 ng;ml every 48 hours, hatched bars; 3 ng;ml every 48 hours, black bars; on ratios of cortisol concentration to concentrations of dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEAS), 17-hydroxyprogesterone (17OH-P), androstendione (andro), and 11-deoxycortisol (11-deoxyc) in ACTH-stimulated cultures of human fetal adrenals during the third week of culture. Values are percentages of controls (cultures with no rTNF; white bars) and means (+;− SD) of 4 different experiments. (P < 0.001 in all cases;as compared to control).*

in the fetoplacentary unit have an important role in directing the steroidogenesis of the fetal adrenals towards increased production of precursors for the placental hormone synthesis. The origin of the TNF in the decidual and placental tissues and in the adrenals remains unclear. At least in the former tissue homogenates the addition of LPS, which is known to be a potent stimulator of TNF from monocyte-macrophages, had no effect on the levels of TNF detected (Jäättelä *et al.*, 1988). This argues somewhat against the role of monocyte-macrophages as the source, although these cells are known to be regular components of the tissues in question (Bulmer and Johnson, 1984: Hume *et al.*, 1984), unless they are already maximally stimulated *in vivo*. Since high affinity receptors for TNF are present in almost all tissues, local production seems more likely than systemic production followed by selective distribution in these tissues. The analysis of the cellular origin of TNF is in process using radioactive probes to detect TNF mRNA in *in situ* hybridization experiments. A remote possibility is also that TNF might play a role in the programmed regression of the proportionally very large fetal adrenals immediately after birth.

Acknowledgements

The technical assistance of Maija-Liisa Mäntylä, Riitta Sissonen and Björn Lindroos is gratefully acknowledged. All the fetuses were obtained from I and II Department of Gynecology and Obstetrics and adult adrenals from II Department of Surgery, University of Helsinki. This work was supported by the Finnish Cultural Foundation and the Farmos Foundation.

References

AGGARWAL, B.B., KOHR, W.J., HASS, P.E., MOFFAT, B., SPENCER, S.A., HENZEL, W. J., BRINGMAN, T.S., NEDWIN, G.E., GOEDDEL, D.V. and HARKINS, R.N. (1985). Human tumor necrosis factor. Production, purification, and characterization. *J. Biol. Chem. 260:* 2345-2354.

BEUTLER, B. and CERAMI, A. (1988). Tumor necrosis factor, cachexia, shock and inflammation: a common mediator. *Annu. Rev. Biochem. 57:* 505-518.

CARSWELL, E.A., OLD, L.J., KASSEL, R.L., GREEN, S., FIORE, N. and WILLIAMSON, B. (1975). An endotoxin-induced serum factor that causes necrosis of tumors. *Proc. Natl. Acad Sci. USA 72:* 3666-3670.

CERAMI, A., IKEDA, Y., LE TRANG, N., HOTEZ, P.J. and BEUTLER, B. (1985). Weight loss associated with an endotoxin-induced mediator from peritoneal macrophages: the role of cachectin (tumor necrosis factor). *Immunol. Lett. 11:* 173-177.

CORNELIUS, P., ENERBACK, S., BJURSELL, G., OLIVECRONA, T. and PEKALA, P.H. (1988). Regulation of lipoprotein lipase mRNA content in 3T3-L1 cells by tumor necrosis factor. *Biochem. J. 249:* 765-769.

JÄÄTTELÄ, M., CARPEN, O., STENMAN, U-M. and SAKSELA, E. Regulation of ACTH-induced steroidogenesis in primary cultures of human fetal adrenals by recombinant tumour necrosis factor type alpha. (Submitted).

JÄÄTTELÄ, M., KUUSELA, P. and SAKSELA, E. (1988). Demonstration of tumor necrosis factor in human amniotic fluids and supernatants of placental and decidual tissues. *Lab. Invest. 58:* 48-52.

KRÖNKE, M., SCHLUTER, C. and PFIZENMAIER, K. (1987). Tumor necrosis factor inhibits MYC expression in HL-60 cells at the level of mRNA transcription. *Proc. Natl. Acad. Sci. USA 84:* 469-473.

PEKALA, P.H., KAWAKAMI, M., ANGUS C.W., LANE, M.D. and CERAMI, A. (1983). Selective inhibition of synthesis of enzymes for *de novo* fatty acid biosynthesis by an endotoxin-induced mediator from exudate cells. *Proc. Natl. Acad. Sci. USA 80:* 2743-2747.

SELIGER, B., STARK, G. and PFIZENMAIER, K. (1988). Tumor necrosis factor-α affects LTR-controlled oncogene expression in transformed mouse fibroblasts at the post-transcriptional level. *J. Immunol. 141:* 2138-2144.

VILLEE, D.B. (1972). The development of steroidogenesis. *Am. J. Med. 53:* 533-544.

VOUTILAINEN, R. and KAHRI, A. (1980). Placental origin of the suppression of 3β-hydroxysteroid dehydrogenase in the fetal zone cells of human fetal adrenals. *J. Steroid Biochem. 13:* 39-43.