Interleukin-1 stimulates tumor necrosis factor-α (TNF-α) release from cytotrophoblastic BeWo cells independently of induction of the TNF-α mRNA

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Received 5 December 1996; revised version received 12 February 1997

Abstract Constitutive tumor necrosis factor-α expression (TNF-α) has been detected in first trimester trophoblast cells and differentiated syncytiotrophoblasts. However, molecules which induce TNF-α release from these cells and their mechanism of action have not been defined. We show for the first time that interleukin-1 (IL-1), a regulator of trophoblast development, induces TNF-α expression in proliferating cytotrophoblastic cells and purified term trophoblasts. Both IL-1α and β stimulate TNF-α release from BeWo cells and TNF-α mRNA was transiently expressed. In growth-arrested/differentiated BeWo cells TNF-α mRNA was detectable without inducer, however, in the presence of IL-1β TNF-α secretion was weakly stimulated compared to proliferating cells. Cycloheximide strongly increased IL-1β-induced TNF-α mRNA concentration indicating that de novo protein synthesis is not required for TNF-α gene expression. However, treatment with cycloheximide did not prevent IL-1β-stimulated release of TNF-α, indicating that the cytokine can regulate TNF-α secretion at a posttranslational level, independently of TNF-α mRNA induction. Besides demonstration of this novel mechanism of IL-1-stimulated TNF-α expression, our data indicate an important role of IL-1 in TNF-α production of cytotrophoblastic cells.

Key words: Interleukin-1; Cytotrophoblastic cell; Tumor necrosis factor-α expression

1. Introduction

Tumor necrosis factor-α (TNF-α), originally identified in macrophages, is a proinflammatory cytokine which exhibits pleiotropic effects on cells. TNF-α not only inhibits growth of certain tumors, but alters proliferation, differentiation and metabolism of a variety of cells (reviewed in [1]). The role of TNF-α in various physiological processes was demonstrated, ranging from inflammation and protection against infections to embryogenesis and hematopoiesis [2,3]. Besides its physiological roles TNF-α was shown to be involved in a variety of diseases and was described as the major mediator of septic shock [4]. The biological properties of the protein are signalled through two distinct receptors (p55 and p75) which mediate their responses via different signal transducers [5].

Within the reproductive tract the influence of TNF-α on pregnancy-specific hormone expression has been demonstrated. TNF-α was shown to affect progesterone, estradiol and human chorionic gonadotropin (hCG) production in placental fragments and JEG-3 choriocarcinoma cells [6]. Moreover, mouse placental lactogen II expression in trophoblast cells was selectively inhibited by TNF-α through TNF-α type I receptor [7].

Endogenous uterine TNF-α expression was originally detected in human decidua and the amniotic fluid [8,9] and in the murine placenta [10]. In pregnant rats TNF-α mRNA was shown to be localized in uterine epithelial, decidual and trophoblast giant cells as well as in placental macrophages. In the latter, however, immunoreactive TNF-α could not be identified which suggested that macrophages do not contribute to placental TNF-α production under normal physiological conditions [11]. In the human placenta, TNF-α mRNA and protein [12] and a low-affinity TNF receptor [13] were shown to be expressed in placental syncytiotrophoblasts. By in situ hybridization TNF-α transcripts were also detected in cytotrophoblast cells isolated from early gestation tissue and TNF-α protein was immunocytochemically identified in choriocarcinoma cells used as a model for proliferating trophoblasts [14]. However, secretion of TNF-α from growing choriocarcinoma cells [14] was either low (Jar cells) or undetectable (JEG-3 cells) suggesting that in vivo additional factors might be required for TNF-α release from cytotrophoblasts.

Therefore, we were interested to evaluate whether interleukin-1 (IL-1), which is known to play an important role in placentation, might stimulate TNF-α expression in trophoblast cells. In this paper we demonstrate that IL-1 induces TNF-α secretion from proliferating choriocarcinoma cells as well as from purified villous trophoblasts isolated from term placenta. Moreover, we show that IL-1β enhances TNF-α mRNA expression in BeWo cells and stimulates release of preformed TNF-α protein by an independent mechanism.

2. Materials and methods

2.1. Isolation of term trophoblast cells

Term trophoblast cells were isolated from selected placental tissue between 38 and 41 weeks of gestation after spontaneous delivery or cesarean section. Briefly, tissue was subjected to two trypsin (0.125%)/DNase I (0.16 mg/ml) digestions. After sedimentation of tissue particles, cell suspension from the supernatant was fractionated on a 5–70% discontinuous Percoll gradient (Pharmacia Biotech, Uppsala, Sweden) as previously described [15]. To remove remaining leukocytes trophoblast cells were further incubated with a CD45 antibody coupled to magnetic beads (Dynal, Oslo, Norway). Purity of trophoblasts was determined on cytospins by counting positive cells after immunocytochemical detection with an anticytokeratin antibody as described [16]. Cells with a purity of 85–90% were cultivated on 24-well plates in DMEM supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 IU/ml penicillin, 100 μg/ml streptomycin) in a humidified incubator with 5% CO_2 and 95% air. Cells were seeded at a density of 1×10^5 cells/cm^2. After 24 h in culture, medium was changed and human recombinant interleukin-1 (R and D Systems, Abingdon, UK) was added to a final concentration of 2.5 ng/ml.

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PII S0014-5793(97)00190-7

2.2. Cultivation of choriocarcinoma cells

Choriocarcinoma cell lines were obtained from American Type Culture Collection (Rockville, MD). BeWo choriocarcinoma cells (ATCC CCL-98) were grown in DMEM supplemented with 10% FBS and antibiotics (100 IU/ml penicillin, 100 μg/ml streptomycin). JAR choriocarcinoma cells (ATCC HTB-144) were cultured in RPMI 1640 medium with the same supplements. Cells were maintained under standard tissue culture conditions in a humidified incubator with 5% CO_2 and 95% air. For treatment with cytokines, cells were seeded at a density of 2×10^5 cells/cm^2. After one additional day in culture fresh medium containing human recombinant IL-1α or IL-1β was added. For induction of growth arrest/differentiation cells were treated with 1 μM methotrexate (MTX, Sigma Chemical Co., St. Louis, MO, USA) added to the growth medium. Induction of TNF-α release was performed by addition of 2.5 ng/ml IL-1β, 48 h after addition of MTX. To inhibit protein synthesis, BeWo cells were incubated for 2 h in the presence of 10 μg/ml cycloheximide (Sigma Chemical Co.) prior to the addition of IL-1β.

2.3. Measurement of TNF-α from cell culture supernatants

Supernatants were aspirated from cell cultures and frozen on dry ice after various times of incubation with IL-1α or β. TNF-α concentration was determined twice in each sample by a one-step 'sandwich' enzyme immunoassay (Immunotech, Marseille, France) according to the instructions of the manufacturer. The sensitivity of the assay in culture supernatant was limited to 10 pg/ml. As specified by the supplier, there is no detectable cross-reactivity with either TNF-β or different forms of TNF receptors.

2.4. Isolation and hybridization of RNA

Total RNA was isolated from cells by direct lysis within the culture flask using TRI Reagent (Molecular Research Center Inc., Cincinnati, OH, USA) according to the manufacturer's instructions. For Northern blot analyses, equal amounts of RNA (20 μg) were glyoxylated and separated by electrophoresis on agarose gels as described [17]. The fractionated RNA was transferred from gels to nylon membranes (Gene Screen, DuPont NEN), crosslinked to the membrane by UV irradiation, hybridized to the ^{32}P-labelled TNF-α cDNA (ATCC 53007), washed and exposed to X-ray films. Radiolabelling of cDNA was performed using the Megaprime DNA labelling system according to the instructions of the manufacturer (Amersham, Buckinghamshire, UK). All hybridizations were performed in 50% formamide, 5×SSC, 1×Denhardt's solution, 50 mM sodium phosphate (pH 6.5) and 200 μg/ml single-stranded salmon sperm DNA for 24 h at 42°C. The last post-washes were in 0.2×SSC/0.1% SDS at 65°C. Filters were stripped and rehybridized with a ^{32}P-labelled glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA fragment. Autoradiographs were densitometrically scanned and quantification of TNF-α mRNA signals was done with Pdi Analysis Software for Biological Data. Hybridization signals of TNF-α were normalized according to the GAPDH signals.

3. Results

Upon addition of human recombinant IL-1β into the culture medium of choriocarcinoma cells and isolated term trophoblasts we observed a time-dependent increase of TNF-α within the supernatant. In proliferating cytotrophoblastic cells TNF-α release was rapidly induced. 6 h after the addition of 2.5 ng IL-1β we detected 360 pg/ml and 393 pg/ml TNF-α in the culture medium of BeWo and Jar cells, respectively (Fig. 1). Compared to the choriocarcinoma cell lines we observed a slightly different kinetics of TNF-α release from purified term trophoblast cells and we only detected 248 pg/ml TNF-α 18 h after IL-1β addition (Fig. 1). We have also measured TNF-α secretion from BeWo, Jar and term trophoblasts in the absence of IL-1β at different times in culture. However, we could not detect quantifiable amounts of the protein suggesting that in non-stimulated cells TNF-α levels were below the detection limit of the enzymatic TNF-α assay (≤10 pg/ml). In BeWo and Jar choriocarcinoma cells TNF-α

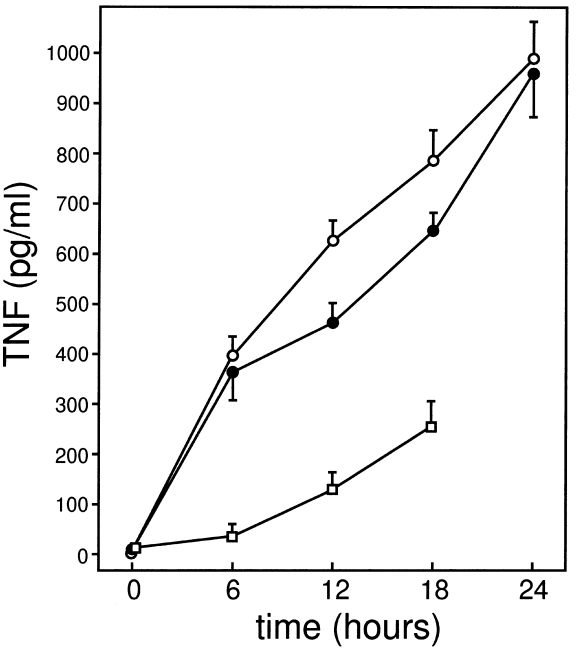

Fig. 1. Stimulation of TNF-α secretion upon addition of IL-1β. BeWo and Jar choriocarcinoma cells were seeded in T25 flasks at a density of 2×10^5 cells/cm^2 and cultured for an additional 24 h before induction with 2.5 ng/ml human recombinant IL-1β. Term trophoblasts were purified as described in Section 2, seeded at a density of 1×10^5/cm^2 and treated with 2.5 ng/ml IL-1β. Cell culture supernatants were isolated after 0, 6, 12, 18 and 24 h of stimulation and TNF-α concentration was determined by ELISA. Open and filled circles indicate TNF-α values obtained from Jar and BeWo cells, respectively. Open squares represent values measured in supernatants from purified term trophoblasts. Values are the mean from three independent experiments, error bars represent S.D.

secretion was further increased up to 1 ng/ml when cells were incubated for 24 h in the presence of IL-1β (Fig. 1).

TNF-α release from BeWo cells was induced by IL-1α as well as IL-1β in a dose-dependent manner. Addition of 10 pg/ml of human recombinant IL-1α or IL-1β to the culture medium induced quantifiable amounts of TNF-α after 8 h of stimulation (Fig. 2A) and 10 ng/ml of each cytokine strongly enhanced TNF-α release. However, IL-1β was found to be more effective than IL-1α, at least in the range of 0.1–1 ng/ml. Furthermore, we investigated the time-dependent induction of TNF-α secretion in BeWo cells treated with the same amounts of either IL-1α or IL-1β (Fig. 2B). Consistently, TNF-α values quantitated from IL-1β-stimulated cells were higher than those measured in the supernatants isolated from IL-1α-incubated cells indicating that IL-1β was indeed the better inducer.

To investigate the molecular mechanism of IL-1-induced TNF-α expression in more detail, we performed Northern transfer of RNA isolated from IL-1β-stimulated and non-stimulated BeWo cells and hybridization with the ^{32}P-labelled TNF-α cDNA (Fig. 3). While TNF-α transcripts were undetectable in total RNA from non-induced cells, we observed a 1.9 kb transcript in IL-1β-stimulated cells after a short exposure to films. Hybridization was specific to the TNF-α mRNA because the ^{32}P-labelled cDNA detected the TNF-α transcript from lipopolysaccharide-stimulated U937 cells (data not shown). After rapid induction TNF-α mRNA abundance decreased 3.5-fold within 4 h (Fig. 3) and was almost undetect-

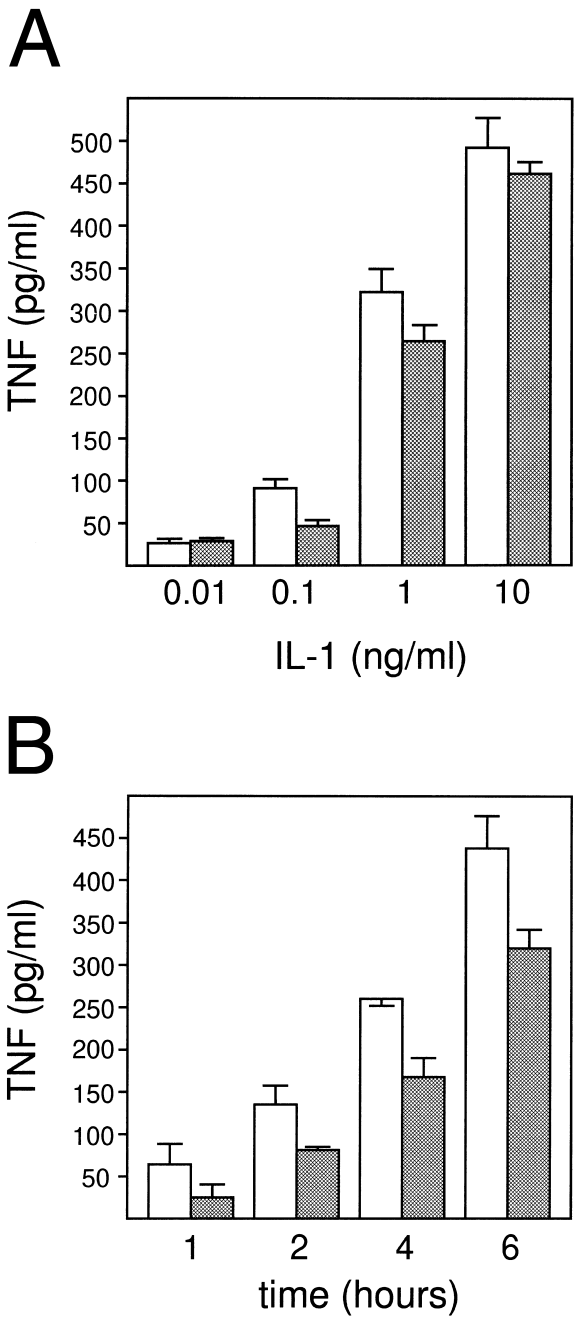

Fig. 2. A: IL-1α and IL-1β induce TNF-α release in a dose-dependent manner. BeWo cells were seeded at a density of 2×10^5 cells/cm^2 and incubated with different amounts of recombinant human IL-1α (hatched bars) or IL-1β (open bars). After 8 h supernatants were isolated and TNF-α concentration was determined by enzyme immunoassay. Bars represent the range in two experiments. B: Time-dependent TNF-α release upon addition of IL-1α or IL-1β. BeWo cells, seeded at a density of 2×10^5 cells/cm^2, were incubated with 3 ng of human recombinant IL-1α or IL-1β. Cell culture supernatants were aspirated after 1, 2, 4 and 6 h and TNF-α concentration was determined as described above. Hatched and open bars indicate TNF-α values after stimulation with IL-1α or IL-1β, respectively. Bars represent the range in two experiments.

able after 18 h of incubation (not shown). Usually, peak levels of TNF-α mRNA were observed between 1 and 2 h after induction depending on the experiment. Conversely, TNF-α concentrations in the supernatant of the same cells steadily increased up to 318 pg/ml after 4 h of incubation.

To determine whether de novo protein synthesis might be required for TNF-α mRNA expression we performed IL-1β induction after treatment with cycloheximide, a potent inhibitor of protein translation. BeWo cells were preincubated for 2 h in the presence of cycloheximide and, subsequently, either stimulated or not stimulated with the cytokine (Fig. 4). Incubation of cells with cycloheximide alone resulted in an increase of TNF-α mRNA; however, active TNF-α was not released, since the protein could not be detected in the supernatant. Addition of IL-1β to translationally inhibited cells strongly induced TNF-α mRNA expression indicating that the induction of TNF-α transcripts was independent of new protein synthesis. Interestingly, secretion of TNF-α was not abolished under those conditions. After 6 h of incubation with IL-1β we detected 248 pg/ml and 372 pg/ml of TNF-α in cycloheximide-treated and untreated cells, respectively. Continuous TNF-α release in the presence of cycloheximide and IL-1β was also observed in a second, independent experiment (not shown).

Finally, we analyzed the influence of growth arrest and/or differentiation on IL-1-inducible TNF-α release. BeWo cells were incubated in the presence of MTX and then incubated in the presence of IL-1β. Cells which had received the drug for 48 h did not divide in culture, since we could not detect an increase in cell number relative to the starting culture. However, cells were viable, as was estimated by trypan blue exclu-

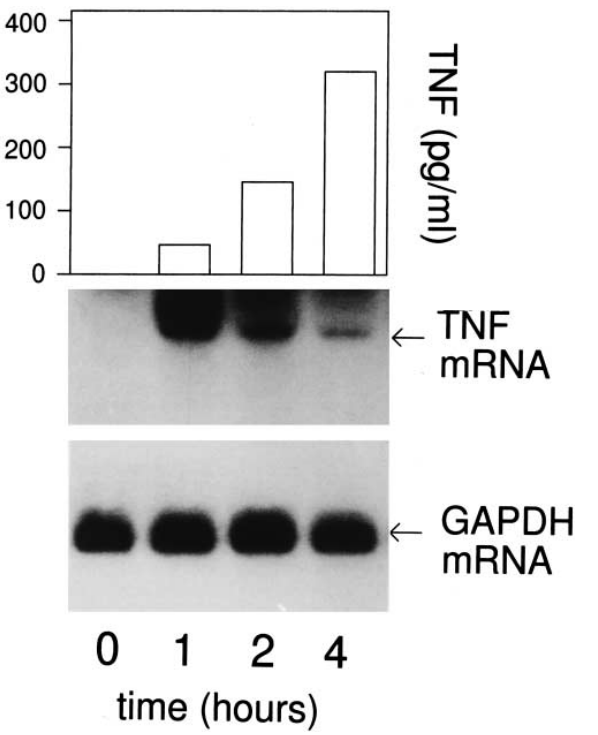

Fig. 3. TNF-α mRNA expression in IL-1β-induced BeWo cells. BeWo cells were incubated in the presence of 2.5 ng/ml human recombinant IL-1β. Supernatants were aspirated from cells 0, 1, 2, and 4 h after IL-1β addition and total RNA was prepared as described in Section 2. TNF-α mRNA expression was visualized by hybridization with the ^{32}P-labelled TNF-α cDNA. Quantification of signals was performed by densitometric scanning of the autoradiographs. TNF-α values were normalized to GAPDH hybridization in each sample. TNF-α release was quantitated from supernatants by ELISA. TNF-α and GAPDH mRNA (arrows) and TNF-α concentration in the medium (open bars) are indicated.

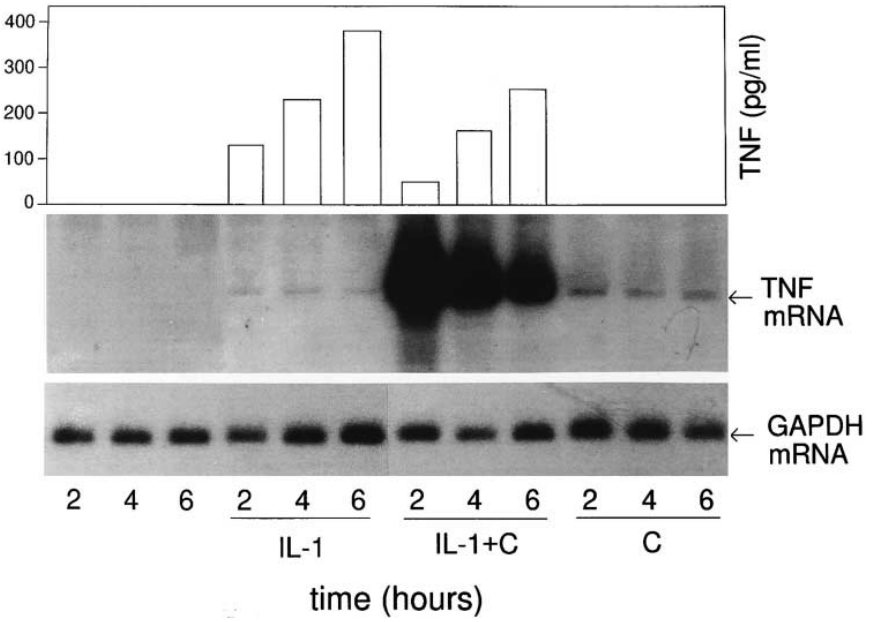

Fig. 4. The influence of cycloheximide on Il-1β-induced TNF-α mRNA expression and protein release. BeWo cells were treated for 2 h with 10 μg/ml cycloheximide (C) and then incubated with or without 2.5 ng/ml IL-1β. As a control, cells which did not receive cycloheximide treatment were stimulated with the same amount of IL-1β. Cell supernatants and RNA were isolated after 2, 4, and 6 h of incubation. TNF-α protein in the medium (open bars) and TNF-α and GAPDH mRNA were analyzed as described above.

sion, and hCG secretion, measured by RIA, was 3.5-fold induced suggesting functional differentiation (data not shown). Contrary to proliferating cells, MTX-treated cells displayed TNF-α mRNA expression and protein secretion without any inducer (Fig. 5). When IL-1β was added TNF-α mRNA expression and TNF-α release were enhanced in both growing as well as non-growing cells. In proliferating cells the TNF-α concentration rose to 182 pg/ml within 2 h while in differentiated cells 319 pg/ml was detected. However, the relative induction in MTX-treated cells was only 2.2-fold between 0 and 2 h suggesting that inducible secretion of the cytokine was downregulated in resting cells.

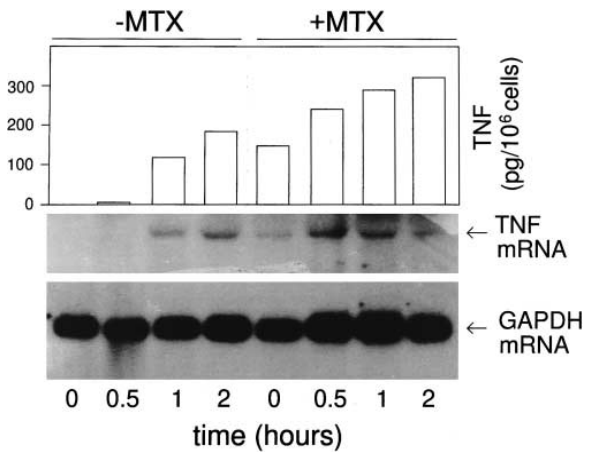

Fig. 5. Inducible TNF-α mRNA expression and secretion in proliferating and growth-arrested cells. BeWo cells were seeded at a density of 4×10^5 cells/cm^2. Before reaching confluency medium with or without 1 μM methotrexate was added. Cells were cultivated for an additional 48 h and 2.5 ng/ml recombinant IL-1β was added. Cell culture supernatants as well as RNA were isolated after 0, 0.5, 1 and 2 h and analyzed as described above. To compare the release of active TNF-α from proliferating and differentiated cells, TNF-α protein concentrations (open bars) were normalized to the individual cell number of MTX-treated and non-treated cultures.

4. Discussion

TNF-α was found to be expressed in cytotrophoblasts, choriocarcinoma cells and syncytiotrophoblasts [12,14], and its role in placental hormone secretion has been demonstrated [6,18]. However, to our knowledge, factors which actually stimulate the release of TNF-α from trophoblast cells were not described. Therefore, we have investigated TNF-α expression in the presence of IL-1β, another cytokine which is involved in the trophoblast-differentiation process. IL-1β has been detected in maternal decidua during pregnancy [19,20] and was shown to stimulate aromatase activity [21], secretion of hCG from cytotrophoblasts [22], and metalloproteinase activity, thereby modulating trophoblast invasion [23]. Here, we demonstrate that IL-1β efficiently induces TNF-α release from proliferating BeWo and JAR choriocarcinoma cells and purified term trophoblasts in culture. Both IL-1α and β stimulate TNF-α secretion with doses as low as 10 pg/ml, suggesting that IL-1 could also be an important regulator of TNF-α expression in vivo. Secreted TNF-α protein might control proliferation of cytotrophoblasts by positive feedback on the TNF-p60 receptor, which is required for ongoing DNA synthesis in cytotrophoblastic cells [14]. On the other hand, IL-1β was shown to decrease proliferation of cultured BeWo cells in a dose-dependent manner [24] which might be explained by the cytostatic effects of TNF-α. Indeed, TNF-α was shown to induce apoptosis in primary cytotrophoblasts and syncytiotrophoblasts and a role in the regulation of trophoblast turnover has been suggested [25,26]. In vivo, however, trophoblast cells are exposed to a complex mixture of cytokines and their antagonists. Therefore, the cytotoxic effects of TNF-α might be

counteracted by soluble TNF receptors which are efficiently secreted from first trimester trophoblastic villi [27].

Expression of the human TNF-α gene was shown to be regulated at multiple levels, including gene transcription, turnover of TNF-α mRNA and protein processing (reviewed in [28]). The TNF-α promoter contains several recognition sequences for NF-κB [29] but depending on cell type and stimulus, distinct combinations of binding sites as well as different factors are required for transcriptional activation [30]. IL-1-induced increase of IL-6 mRNA is performed by NF-κB activation, which has also been implicated in IL-1-mediated TNF-α gene transcription [31].

TNF-α mRNA production has been detected in poly A^+ RNA from choriocarcimona cells [14]; however, in total RNA isolated from non-stimulated BeWo cells we did not observe TNF-α transcripts after short exposure suggesting that constitutive mRNA expression is low. Upon addition of IL-1β TNF-α mRNA was easily detectable under our experimental conditions and we observed a transient TNF-α mRNA expression which is typical of NF-κB-activated genes [32]. Although we have not measured gene transcription directly, we demonstrate that IL-1-induced increase of TNF-α mRNA does not require de novo protein synthesis, suggesting that in trophoblast cells TNF-α transcription might be induced by the well described posttranslational activation of NF-κB and/or NF-κB-like molecules [33]. On the other hand, TNF-α gene transcription was shown to be repressed by short-lived DNA-binding proteins [34]. IL-1β might therefore facilitate transcription by inactivating these repressors.

We found that TNF-α mRNA expression was highly upregulated in the presence of cycloheximide and IL-1β. Superinduction of TNF-α mRNA has been explained by translational inhibition of a degrading RNase which mediates the rapid turnover of the transcript via destabilizing elements in the 3′ region of the mRNA [35]. Indeed, in BeWo cells we observed TNF-α mRNA induction after cycloheximide treatment in the absence of IL-1β. Therefore, we assume that the strong expression of TNF-α mRNA upon addition of both substances was due to the combined effect of cycloheximide-induced stabilization of transcripts and IL-1-induced transcriptional activation.

Interestingly, there is only a minor contribution of newly synthesized TNF-α mRNA to the overall TNF-α expression, since inducible TNF-α release was not abolished in cytotrophoblastic cells which had been translationally inhibited 2 h before incubation with IL-1β. This result indicates that IL-1β induces TNF-α secretion of constitutively synthesized TNF-α protein without a requirement for new protein translation and independently of stimulation of TNF-α mRNA expression. This might by accomplished by posttranslational activation of protease(s) which process the membrane-anchored pro-TNF-α to the mature, secreted form by internal protein cleavage [36,37].

Finally, we demonstrate that growth arrested/differentiated BeWo cells express higher levels of TNF mRNA and secreted protein than proliferating cells. However, TNF-α release was found to be less inducible upon IL-1β treatment. This result suggests that IL-1 type 1 receptors which were detected on early gestation trophoblasts [23] might be downregulated during trophoblast differentiation but further studies are needed.

In summary, we demonstrate for the first time that IL-1 is a potent inducer of TNF-α in cytotrophoblastic cells. Accordingly, it is tempting to speculate that IL-1β, which is highly expressed in first trimester trophoblasts but produced at low levels in the third trimester [23], might also control TNF-α expression in vivo and thereby influence trophoblast differentiation and/or turnover. On the other hand, in infected fetal membranes, IL-1 released from placental macrophages, trophoblasts or other sources might induce secretion of large amounts of trophoblast-derived TNF-α. This may enhance inflammatory reactions as well as cytotoxic effects of TNF-α on the trophoblast.

Acknowledgements: We thank W. Lajta, R. Kuzmits and U. Tretzmüller for technical assistance, especially for preparation of purified term trophoblast cells.

References

[1] Aggarwal, B.B. and Natarajan, K. (1996) Eur. Cytokine Netw. 7, 93–124.
[2] Wride, M.A. and Sanders, E.J. (1993) Dev. Dyn. 198, 225–239.
[3] Jacobsen, S.E.W., Ruscetti, F.W., Dubois, C.M. and Keller, J.R. (1992) J. Exp. Med. 175, 1759–1772.
[4] Beutler, B., Milsark, I.W. and Cerami, A.C. (1985) Science 229, 869.
[5] Tartaglia, L.A. and Goeddel, D.V. (1992) Immunol. Today 13, 151–153.
[6] Pedersen, A.M., Fulton, S.K., Porter, L. and Francis, G.L. (1995) J. Reprod. Immunol. 29, 69–80.
[7] Yamaguchi, M., Kurachi, H., Tadokoro, C., Sakata, M., Yoshimoto, Y., Masumoto, N., Tasaka, K. and Miyake, A. (1994) Biochem. Biophys. Res. Commun. 202, 1599–1605.
[8] Casey, M.L., Cox, S.M., Beutler, B., Milewich, L. and McDonald, P.C. (1989) J. Clin. Invest. 83, 430–436.
[9] Jaattela, M., Kuusela, P. and Saksela, M.E. (1988) Lab. Invest. 58, 48–52.
[10] Crainie, M., Guilbert, L. and Wegmann, T.G. (1990) Biol. Reprod. 43, 999–1005.
[11] Yelavarthi, K.K., Chen, H.-L., Yang, Y., Cowley Jr., B.D., Fishback, J.L. and Hunt, S.J. (1991) J. Immunol. 146, 3840–3848.
[12] Chen, H.-L., Yang, Y., Hu, X.-L., Yelavarthi, K.K., Fishback, J.L. and Hunt, J.S. (1991) Am. J. Pathol. 139, 327–335.
[13] Hampson, J., McLaughlin, P.J. and Johnson, P.M. (1993) Immunology 79, 485–490.
[14] Yang, Y., Yelavarthi, K.K., Chen, H.-L., Pace, J.L., Terranova, P.F. and Hunt, J.S. (1993) J. Immunol. 150, 5614–5624.
[15] Kliman, H.J., Nestler, J.E., Sermasi, E., Sanger, J.M. and Strauss III, J.F. (1986) Endocrinology 118, 1567–1581.
[16] Kiss, H., Stefaner, I., Neuwirth, S., Reisenberger, K., Ellinger, A. and Egarter, Ch. (1996) Geburtsh. Frauenheilkd. 56, Artikel 602, 1–8.
[17] McMaster, G.K. and Carmichael, G.G. (1977) Proc. Natl. Acad. Sci. USA 74, 4835–4838.
[18] Yanushpolsky, E.H., Ozturk, M., Polgar, K., Berkowitz, R.S. and Hill, J.A. (1993) J. Reprod. Immunol. 25, 235–247.
[19] Kauma, S., Matt, D., Strom, S., Eierman, D. and Turner, T. (1990) Am. J. Obstet. Gynecol. 163, 1430–1437.
[20] Romero, R., Wu, Y.K., Broody, D., Oyarzun, E., Duff, G.W. and Durum, S.K. (1989) Obstet. Gynecol. 73, 31–34.
[21] Nestler, J.E. (1993) Endocrinology 132, 566–570.
[22] Yagel, S., Lala, P.K., Powell, W.A. and Casper, R.F. (1989) J. Clin. Endocrinol. Metab. 68, 992–995.
[23] Librach, C.L., Feigenbaum, S.L., Bass, K.E., Cui, T.-Y., Verastas, N., Sadovsky, Y., Quigley, J.P., French, D.L. and Fisher, S.J. (1994) J. Biol. Chem. 269, 17125–17131.
[24] Lewis, M.P., Sullivan, M.F.H. and Elder, M.G. (1994) Placenta 15, 13–20.
[25] Yui, J., Garcia-Lloret, M., Wegmann, T.G. and Guilbert, L.J. (1994) Placenta 15, 819–835.
[26] Yui, J., Hemmings, D., Garcia-Lloret, M. and Guilbert, L.J. (1996) Biol. Reprod. 55, 400–409.
[27] Kelly, R.W., Carr, G.G., Elliott, C.L., Tulppala, M. and Critchley H.O.D. (1995) Hum. Reprod. 10, 3289–3292.

[28] Pauli, U. (1994) Crit. Rev. Eukaryotic Gene Expression 4, 323–344.
[29] Shakhov, A.N., Collart, M.A., Vassalli, P., Nedospasov, S.A. and Jongeneel, C.V. (1990) J. Exp. Med. 171, 35–47.
[30] Tsai, E.Y., Jain, J., Pesavento, P.A., Rao, A. and Goldfeld, A.E. (1996) Mol. Cell. Biol. 16, 459–467.
[31] Bethea, J.R., Chung, I.Y., Sparacio, S.M., Gillespie, G.Y. and Benveniste E.N. (1992) J. Neuroimmunol. 36, 179–191.
[32] LeBail, O., Schmidt-Ullrich, R. and Israel, A. (1993) EMBO J. 12, 5043–5049.
[33] Baeuerle, P.A. and Baltimore, D. (1988) Science 242, 540–546.
[34] Collart, M.A., Belin, D., Vassalli, J.D., de Kossodo, S. and Vassalli, P. (1986) J. Exp. Med. 173, 801–811.
[35] Han, J., Beutler, B. and Huez, G. (1991) J. Immunol. 146, 1843–1848.
[36] Kim, K.U., Kwon, O.J. and Jue, D.M. (1993) Immunology 80, 134–139.
[37] Gearing, A.J.H., Beckett, M., Christodoulou, M., Churchill, R., Clements, J.M., Davidson, A.H., Drummond, A.H., Galloway, W.A., Gilber, R., Gordon, J.L., Leber, T.M., Mangan, M., Miller, K., Nayee, P., Owen, K., Patel, S., Thomas, W., Wells, G., Wood, L.M. and Wooley, K. (1994) Nature 370, 555–557.