Tumor necrosis factor- α inhibits glutathione S-transferase- α expression in cultured porcine Sertoli cells

L Benbrahim-Tallaa, F Boussouar, C Rey¹ and M Benahmed

Unité 407 de l'Institut National de la Santé et de la Recherche Médicale (INSERM U 407), Communication Cellulaire en Biologie de la Reproduction, Faculté de Médecine Lyon-Sud, 69921 Oullins Cedex, France

¹Unité 189 de l'Institut National de la Santé et de la Recherche Médicale (INSERM U 189), Faculté de Médecine Lyon-Sud, 69921 Oullins Cedex, France
(Requests for offprints should be addressed to M Benahmed; Email: benahmed@lsgrsn1.univ-lyon1.fr)

Abstract

Glutathione S-transferases (GSTs) are a family of soluble enzymes of detoxification that use reduced glutathione in conjugation and reduction reactions. Toxic electrophiles are substrates for the GSTs. GST α is expressed at high levels in different tissues such as the testis. Among the different GSTs present in the testis, GST α is specifically expressed in Leydig and Sertoli cells known to be under the control of hormonal and local regulatory factors. The present study investigated the regulatory action of tumor necrosis factor- α (TNF α) on basal and hormone (FSH and testosterone)-stimulated GST α expression in cultured Sertoli cells. Treatment with TNF α (0–20 ng/ml, 48 h) induced a decrease in basal GST α mRNA levels in a dose-dependent manner (fivefold decrease; $P < 0.001$). The maximal and half maximal effects were observed at 20 ng/ml and 7 ng/ml respectively. The inhibitory effect of TNF α was also time-dependent with a maximal inhibitory effect (threefold decrease; $P < 0.001$) observed at 48 h. The inhibitory effect of the cytokine was also observed on basal GST α protein (28 kDa) levels. TNF α also inhibited the hormone-stimulated GST α expression in Sertoli cells. The treatment of cultured Sertoli cells with both FSH and TNF α (100 ng/ml and 10 ng/ml respectively, 48 h) resulted in a complete suppression of the stimulatory action

of FSH on GST α mRNA levels. Similarly, in Sertoli cells treated with testosterone or its non-aromatizable metabolite dihydrotestosterone (100 ng/ml, 24 h), TNF α reduced the hormone-stimulated GST α mRNA and protein levels. TNF α inhibited basal GST α expression without affecting mRNA stability. Indeed, the decay curves (mRNA half-life time = 18 h) for the GST α basal mRNA levels in Sertoli cells was similar in the absence or presence of TNF α (10 ng/ml, 48 h). Testosterone increased GST α mRNA without affecting the enzyme mRNA stability. TNF α antagonized the androgen-stimulated GST α mRNA levels without affecting the enzyme mRNA stability, suggesting that the interaction between the androgen and the cytokine is mostly exerted at a transcriptional level. FSH increased GST α mRNA levels through an increase in mRNA stability (increased mRNA half-life times to 119 h). TNF α antagonized the stimulatory effect of FSH on GST α mRNA levels by antagonizing the stabilizing effect exerted by the hormone on GST α mRNA. Together, these results suggest that the increase in the cytokine levels within the testis would alter the detoxification processes against genotoxic products during spermatogenesis.

Journal of Endocrinology (2002) **175**, 803–812

Introduction

The different cell types contain a wide variety of enzyme systems that protect them from injury triggered by toxic chemicals. One of these enzyme systems is the family of glutathione S-transferases (GSTs; EC 2.5.1.18). This gene superfamily plays a crucial role in protecting cells by using reduced glutathione (GSH; γ -Glu-Cys-Gly) to conjugate many different reactive electrophiles, including drugs, chemotherapeutic agents and carcinogens, leading generally to their inactivation and elimination (Hays & Strange 1995, Seidegard & Ekstrom 1997, Salinas & Wong 1999). In addition to their ability to catalyze the formation of

conjugates, GSTs can also serve as peroxidases and isomerases (Mannervick & Danielson 1988). Both cytosolic and microsomal forms of GSTs have been identified (Hays & Mantle 1986, Papp *et al.* 1995). The cytosolic GSTs exist as homodimers or heterodimers and are separated into several classes (α , μ , π , σ , and θ) based on the degree of homology of their subunit sequences (Pickett & Lu 1989). GST distribution is tissue and sex specific (Tu *et al.* 1983, Listowsky *et al.* 1998); GST expression is under developmental control. The expression of the enzymes is also responsive to physiological stress and inducible by many drugs and chemicals. The structural diversity of the compounds that increase the expression of GSTs suggests

that several distinct mechanisms might be responsible for gene induction (Hays & Pulford 1995).

GST α activity is expressed at high levels in different tissues such as the testis (Gandy *et al.* 1996, Mueller *et al.* 1998). Different GST isoenzymes have been identified in the male gonad. Specifically, GST α (Aceto *et al.* 1989, Sundberg *et al.* 1993) and GST π (Yoganathan *et al.* 1989) have been identified in testicular somatic cells and particularly in Leydig and Sertoli cells. We have previously reported on the expression of GST α in Leydig and Sertoli cells and have shown that, in Sertoli cells, GST α expression is under the positive control of follicle-stimulating hormone (FSH) and androgens (Benbrahim-Tallaa *et al.* 2002). In the context of Sertoli cell-germ cell interactions which support spermatogenesis, Sertoli cells are known to be under endocrine control but also under a local control resulting from growth factor and cytokine production and action in the testis (Benahmed 1996, Griswold 1995, Gnassi *et al.* 1997). Among these factors, we have identified here tumor necrosis factor- α (TNF α) as a potent inhibitor of basal and hormone-stimulated GST α expression in Sertoli cells.

Materials and Methods

Materials

Dulbecco's modified Eagle's medium (DMEM)/Ham's F12 medium and TRizol were obtained from Life Technologies (Eragny, France). Collagenase/dispase was obtained from Roche (Mannheim, Germany). TNF α was purchased from Preprotech (Rocky Hill, NJ, USA). Testosterone, dihydrotestosterone (DHT), 8-bromo-adenosine 3',5'-monophosphate, 5,6-dichlorobenzimidazole riboside (DRB), 3-cyclohexylamino-1-propanesulfonic acid (CAPS), protease inhibitor cocktail and BSA were purchased from Sigma Chemical Company (St Louis, MO, USA). Ovine FSH was kindly provided by Dr A F Parlow of the US Department of Agriculture, Agricultural Research Service, Animal Hormone Program (Beltsville, MD, USA). Rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe was a generous donation by Dr J M Blanchard (Pierre et Marie Curie University, Paris, France). The rabbit polyclonal anti-human GST α antibody (Mehta *et al.* 1994, Murray *et al.* 1995) (NCL-GST α) was purchased from Novocastra-TEBU (Le Perray en Yvelines, France). Peroxidase-conjugate goat anti-rabbit immunoglobulin G was obtained from COVALAB (Lyon, France). Antibody diluent was obtained from DAKO (Copenhagen, Denmark). The Ultravision Detection System was obtained from Lab Vision Corporation (Fremont, CA, USA). The Bradford reagents and Pierce detection kit were purchased from Bio-Rad Laboratories, Inc. (Richmond, CA, USA).

Sertoli cell isolation and culture

Sertoli cells were isolated from immature porcine testes (2–3 weeks old) using collagenase treatment as described by Mather & Philips (1984). Briefly, the testes were decapsulated, minced, and washed in DMEM/Ham's F12 (1:1) medium. After collagenase dissociation (0.4 mg/ml, 90–120 min at 32 °C), cells were washed by mild centrifugation (200 g, 10 min). After 5 min of sedimentation, the tubules were recovered and washed three times by unit gravity in DMEM/Ham's F12 medium. Contaminating interstitial Leydig cells were released after 20 min of treatment (at room temperature) with 20 ml 1 M glycine, 2 mM EDTA, and 20 IU/ml DNase in Ca²⁺, Mg²⁺-free phosphate-buffered saline (pH 7.2). Tubules were then washed three times in DMEM/Ham's F12 medium by gravity before incubation in DMEM/Ham's F12 medium containing collagenase (0.4 mg/ml) and DNase (0.05 mg/ml) for 30 min at 32 °C. The supernatants containing the peritubular myoid cells were removed, and the sedimented tubules were treated again as described above with collagenase (0.4 mg/ml, 30 min, 32 °C) to isolate Sertoli cells. This procedure led to a purified Sertoli cell population free from contamination by either Leydig cells or germ cells and which contained between 2% and 5% peritubular myoid cells. Sertoli cells were further washed several times by gravity in DMEM/Ham's F12. Cells were counted in a Coulter counter (Coulter Electronics, Margency, France), plated in Falcon (Los Angeles, CA, USA) 60 mm Petri dishes (5 × 10⁶ cells/dish), and cultured at 32 °C in a humidified atmosphere of 5% CO₂–95% air in DMEM/Ham's F12 (1:1) medium containing sodium bicarbonate (1.2 mg/ml), 15 mM HEPES, and gentamycin (20 µg/ml). This medium was supplemented with transferrin (5 µg/ml), tocopherol (10 µg/ml), and insulin (2 µg/ml). The culture medium was changed every 2 days and the experiments were carried out in the 2 weeks following cell preparation, during which the cultured Sertoli cells maintained their specific activity. The experiments were carried out from day 4 of culture.

Preparation of total RNA and Northern blotting analysis

To prepare the GST α probe, approximately 600 ng DNA was amplified by polymerase chain reaction (PCR). PCR amplification of the GST α cDNA was carried out using the following primers (Genset SA, Paris, France): forward, 5'-GCTGGCCAACTTCCCTCTGC-3' and reverse, 5'-TGCGTGCGAACAAAACAAAAT-3' and designed to amplify a sequence of 331 base pairs. PCR cycles consisted of initial denaturation of mixed PCR without *Taq* polymerase at 94 °C for 5 min. A total of 30 PCR cycles followed the addition of *Taq* polymerase. Each cycle involved 1 min of denaturation at 92 °C, 1 min of primer annealing at 63 °C, and 1 min of elongation at 70 °C. The

30 cycles were followed by 5 min at 70 °C. The PCR products were separated by electrophoresis in a 1% agarose gel and analysed after ethidium bromide staining and UV illumination. GST α cDNA was purified and then labeled with 50 μ Ci [α -³²P]dCTP using a random priming DNA labeling kit.

Total RNAs from cells in culture were prepared using TRIzol, a monophasic solution of phenol and guanidine isothiocyanate. This reagent is an improvement over the single-step RNA isolation method developed by Chomczynski & Sacchi (1987). Briefly, cells were lysed by adding 1 ml TRIzol reagent and passing the cell lysate through a pipette several times. The homogenized samples were incubated for 5 min to permit the complete dissociation of nucleoprotein complexes. Chloroform (200 μ l) was then added. After centrifugation, the aqueous phase was precipitated with isopropanol (500 μ l), and pellets were washed with 70% ethanol. After solubilization in diethyl pyrocarbonate-treated water, the amount of RNA was estimated by spectrophotometry at 260 nm. About 10 μ g total RNAs, denatured for 15 min at 65 °C in the presence of 2.2 M formaldehyde, 12.5 M formamide and 1 $\times$ 3-N-morpholino propanesulfonic acid (MOPS) were size-fractionated by electrophoresis on 1.2% agarose, 2.2 M formaldehyde gels. After migration in 0.02 M MOPS running buffer, RNAs were transferred to nitrocellulose membrane (Hybond-C extra; Amersham International plc, Amersham, Bucks, UK) by capillary transfer with 10 $\times$ SSC (1.5 M NaCl, 0.15 M sodium citrate) and fixed at 80 °C for 2 h. After 4 h of prehybridization at 42 °C, filters were hybridized with labeled probes (1–4 10^6 c.p.m./ml) overnight at 42 °C in 50% formamide, 5 $\times$ SSPE (0.9 M NaCl, 0.05 M sodium phosphate, 5 mM EDTA, pH 7.5), 5 $\times$ Denharts' solution (1 g Ficoll, 1 g polyvinylpyrrolone, 1 g BSA/l), 1% SDS, and 100 μ g/ml yeast RNA. After this period, membranes were washed four times in 2 $\times$ SSC/0.1% SDS (20 min at room temperature), followed by 40 min at 55 °C. Filters were exposed to Kodak X-OMAT (Eastman Kodak, Rochester, NY, USA) films for 1–2 days at –70 °C. The intensities of the autoradiographic bands were estimated by densitometric scanning using Intelligent Quantifier Software (Bio-Image, Warrington, Cheshire, UK). The data are expressed as the GST α /GAPDH mRNA ratio.

Preparation of total protein and Western blotting analysis

Total proteins were extracted from porcine Sertoli cells treated under different conditions. Briefly, Sertoli cells were homogenized in ice-cold hypotonic buffer (25 mM Tris-HCl, pH 7.4, 1 mM EDTA, and protease inhibitor cocktail). Homogenized cells were centrifuged at 100 000 *g* for 10 min at 4 °C. Protein concentration was determined in the supernatants using the assay of Bradford (1976) with BSA as a standard. Protein extracts (80 μ g/well) were incubated at 100 °C for 5 min under reducing

conditions, then size-fractionated on SDS/polyacrylamide gel. After electrophoresis, the proteins were transferred at a constant voltage of 100 V for 30 min onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany) using 10 mM CAPS (pH 11) containing 10% methanol. Following transfer, the membrane was incubated in a blocking buffer (Tris-buffered saline; TBS) containing 5% non-fat dry milk at 4 °C. The membrane was washed with TBS and stained with Ponceau red. After two washes with TBS, the membrane was then incubated with the rabbit polyclonal anti-GST α antibody diluted 1:1000 in a solution of TBS with 0.5% non-fat dry milk for 2 h at room temperature. After washing with TBS, the membrane was then incubated with the goat anti-rabbit peroxidase-conjugated antibody (Jackson Immunoresearch, West Grove, PA, USA) diluted 1:2000 in TBS buffer with 0.5% non-fat dry milk. The antibody-antigen complexes were detected by chemiluminescence using a Pierce detection kit and autoradiographic material from Bio-Rad.

Statistical analysis

The autoradiographic bands were quantified by densitometric analysis using the Bio Image Scanner (Millipore SA, Saint Quentin, France). Each GST α band was normalized by correcting for any variation in the amounts of RNA applied to each lane. Experiments were repeated at least three times with different cell preparations. Statistical significance of the results was determined by the Student's *t*-test when data from at least three different experiments were compared. Data are presented as the means $\pm$ S.D.

Results

TNF α reduces GST α mRNA and protein levels

The data in Fig. 1 show that in Sertoli cells treated with TNF α there was a dramatic decline in GST α mRNA levels. TNF α decreased GST α mRNA levels in a dose-dependent manner with a maximal effect (fivefold; $P < 0.001$) observed at 20 ng/ml TNF α (Fig. 1A). Such an inhibitory effect was also time-dependent and the maximal effect on GST α mRNA levels was observed after 48 h of treatment with TNF α ($P < 0.001$) (Fig. 1B). The decrease in GST α mRNA levels in TNF α -treated Sertoli cells resulted in a parallel decrease in GST α protein (28 kDa) levels (Fig. 1C).

TNF α antagonizes FSH and testosterone actions on GST α mRNA expression

The interaction between the effects of the hormones (FSH and testosterone) and the cytokine on GST α expression was examined. FSH increased GST α mRNA levels in a

Figure 1 Effect of TNF α on GST α mRNA expression. (A) Sertoli cells were exposed for 24 h to increasing concentrations of TNF α (0–20 ng/ml). (B) Sertoli cells were exposed to TNF α (10 ng/ml) for 0–48 h. Total Sertoli RNA (10 μ g) was subjected to Northern blot analysis with labeled cDNA probe for GST α and GAPDH. (C) Sertoli cells were treated with TNF α (10 ng/ml). Protein extracts (80 μ g) from Sertoli cells were separated on 12% SDS/polyacrylamide gel and transferred onto nitrocellulose and incubated with the polyclonal anti-GST α (1:1000). The complex was revealed with a peroxidase-coupled secondary antibody.

dose-dependent manner with a maximal effect (2.5-fold; $P < 0.002$) observed at 64 ng/ml (Fig. 2A) and the half-maximal (ED_{50}) effect was observed at 14.5 ng/ml FSH. The treatment of Sertoli cells with both FSH (100 ng/ml) and TNF α (10 ng/ml) resulted in a complete suppression of the stimulatory action of FSH on GST α mRNA (Fig. 2B) levels. In Sertoli cells treated with testosterone, GST α mRNA levels increased in a testosterone dose-dependent manner with a maximal effect (twofold; $P < 0.001$) observed at 4 ng/ml testosterone (Fig. 3A) and the ED_{50} effect was observed at 1.8 ng/ml testosterone. The treatment of Sertoli cells with both testosterone (100 ng/ml) and TNF α (10 ng/ml) (Fig. 3B) reduced GST α mRNA levels below control values.

TNF α antagonizes the stimulatory actions of FSH and testosterone on GST α protein levels

The inhibitory effect of TNF α was also observed on GST α protein levels. The treatment of Sertoli cells with FSH (100 ng/ml) and TNF α (10 ng/ml) (Fig. 4A), or with DHT (100 ng/ml) and TNF α (10 ng/ml) (Fig. 4B) resulted in a complete suppression of the stimulatory actions of FSH and DHT on the GST α protein levels.

TNF α antagonizes FSH action on GST α mRNA stability

To decrease GST α mRNA levels, TNF α may act at a transcriptional level and/or by affecting GST α mRNA

Figure 2 Effect of TNF α on FSH action on GST α mRNA. (A) Sertoli cells were exposed for 24 h to increasing concentrations of FSH (0–250 ng/ml). (B) Sertoli cells were treated with FSH (100 ng/ml), TNF α (10 ng/ml) or both for 24 h. Total RNA (10 μ g) was analyzed by Northern blot with [32 P]labeled cDNA probes for GST α and GAPDH. The upper panels show representative autoradiograms; the lower panels show histograms which represent the means $\pm$ S.D. of three experiments.

Figure 3 Effect of TNF α on androgen action on GST α mRNA. (A) Sertoli cells were exposed for 24 h to increasing concentrations of testosterone (0–250 ng/ml). (B) Sertoli cells were treated with testosterone (100 ng/ml), TNF α (10 ng/ml) or both for 24 h. Total RNA (10 μ g) was analyzed by Northern blot with [32 P]labeled cDNA probe for GST α and GAPDH. The upper panels show representative autoradiograms; the lower panels show histograms representing the means $\pm$ S.D. of three experiments.

Figure 4 Effect of TNF α on FSH and androgen action on GST α protein. (A) Sertoli cells were treated with FSH (100 ng/ml), TNF α (10 ng/ml) or both. (B) Sertoli cells were treated with DHT (100 ng/ml), TNF α (10 ng/ml) or both. The protein extracts (80 μ g) were separated on 12% SDS/polyacrylamide gel, transferred onto nitrocellulose and incubated with the polyclonal anti-GST α (1:1000). The complex was revealed with a peroxidase-coupled secondary antibody.

stability. We have tested this second possibility. For this purpose, the transcriptional activity in both control and TNF α -treated Sertoli cells was blocked with 25 μ M DRB, an inhibitor of transcription, and the levels of GST α mRNA were estimated by Northern blotting analysis at various times (6, 12, and 24 h). In the presence of TNF α (Fig. 5A), the decay in Sertoli cell GST α mRNA levels was similar to that of untreated cells (half-life time=18 h) indicating that the cytokine by itself did not affect GST α mRNA stability. In the presence of FSH (100 ng/ml, 48 h), the mRNA levels remained stable (half-life time=111 h) when compared with untreated cells. However, when TNF α (10 ng/ml, 48 h) was added to FSH (100 ng/ml, 48 h), the stabilizing effect of the hormone on GST α mRNA levels was no longer observed (half-life time=24 h). Indeed, the mRNA level of decay in TNF α +FSH-treated cells was comparable with that observed in untreated (control) cells (Fig. 5B). Together, these results indicated that TNF α appears to antagonize FSH at the level of GST α mRNA stability.

The decrease in GST α mRNA levels was similar whether Sertoli cells were treated or not with DHT

(100 ng/ml, 24 h) (Fig. 6A) or with DHT+TNF α (Fig. 6B). These observations indicate that the androgens stimulated GST α expression without affecting GST α mRNA half-life (stability). Together, and as expected, the antagonistic interaction between the androgens and the cytokine did not involve an alteration of GST α mRNA stability.

In control experiments, there were no significant differences in the rate of degradation of GAPDH mRNA in control and pretreated cells.

Discussion

In this report, we have examined the regulatory effect of TNF α on GST α expression in primary cultures of purified Sertoli cells isolated from porcine testes. We present evidence that TNF α reduces GST α protein and mRNA levels in Sertoli cells. The inhibitory effect of TNF α on GST α mRNA appears to be exerted at a transcriptional level since it did not affect GST α mRNA stability and since it was abolished by an inhibitor of transcription, DRB. TNF α reduced GST α mRNA and protein levels in a nanomolar concentration range. Such a concentration is compatible with the amounts of TNF α reported to be present in the microenvironment of Sertoli cells (De *et al.* 1993). In pathological conditions, TNF α , which affects Sertoli cells, may originate from the circulatory system and/or from activated macrophages present in the testicular interstitial tissue (Sawinski *et al.* 1996, Huston 1998). The inhibitory effect of the cytokine on GST α mRNA is probably exerted via a TNF α receptor(s), particularly via the p55 form, which has been reported to be present in Sertoli cells (Riccioli *et al.* 1995, De Cesaris *et al.* 1998). There is now general agreement that TNF α , on binding to its receptors, activates different intracellular signaling pathways, including protein kinase C (PKC), protein kinase A, sphingomyelin metabolites and p38, and activated c-jun N-terminal protein kinase/stress-activated protein kinase (Vilcek & Lee 1991, Boussouar *et al.* 1999, Grataroli *et al.* 2000). It has been shown that TNF α may activate PKC and sphingomyelin metabolite pathways to modulate the expression of lactate dehydrogenase A in Sertoli cells (Boussouar *et al.* 1999, Grataroli *et al.* 2000). Although there is increasing evidence that induction of gene expression by TNF α is mediated through activation of *trans*-acting DNA-binding nuclear proteins, the mechanisms through which TNF α activates nuclear transcription factors potentially involved in GST α mRNA expression are unknown. However, it has been shown that TNF α induced the expression of several target genes through activation of not only a number of transcription factors, including nuclear factor κ B and nuclear factor interleukin-6, but also multiple response factors, including interferon response factor-1, interferon response factor-2, c-Fos, c-Jun, and c-Myc. As previous studies

Figure 5 Effect of TNF α on the FSH-stimulatory effect on GST α mRNA stability. Sertoli cells were incubated (A) in the presence (◆) or absence (□) of TNF α and (B) in the presence of FSH (△) or FSH and TNF α (▲). DRB (25 μ M) was then added to control and treated cells. GST α mRNA levels were evaluated at 0, 6, 12, and 24 h. Total RNA (10 μ g) from each time-point was analyzed by Northern blot with radiolabeled GST α cDNA probe. The data are plotted as the percentage of GST α mRNA remaining relative to that present at time zero. A representative pattern of three separate experiments is shown.

have identified the presence of 12-O-tetraphorbol 12-myristate 13-acetate-responsive elements (Vilcek & Lee 1991) and AP-1 sites (Pickett & Lu 1989) in the GST promoter, it is therefore possible that TNF α regulates GST α transcription through these responsive elements. The GST α expression is markedly inducible by different drugs. The rat GSTA2 gene appears to contain at least four *cis*-acting elements in the 5'-flanking region that respond to xenobiotics. One of these elements is responsible for induction by polycyclic aromatic hydrocarbon and is identical to the xenobiotic-responsive element (Rushmore *et al.* 1990). The second element has been designated the antioxidant-responsive element because it mediates responsiveness to phenolic antioxidants (Rushmore *et al.* 1991, 1993). The third element identified in this gene promoter is identical to the glucocorticoid-responsive element and may render expression of GSTA2 responsive to dexamethasone. The fourth element is responsible for the induction of this gene by barbiturates. Responsiveness to phenobarbital might be mediated by the Barbie box element (Liang *et al.* 1995), and several potential elements were identified in the 5'-flanking region of the rat GSTA2. Friling *et al.* (1990) have also shown that the inducible expression of GSTY α gene is controlled by an electrophilic-responsive element, which is activated exclusively by an electrophilic signal. It has been reported that TNF α has an inhibitory effect on GST μ and pheno-

barbital abrogates this effect in primary cultured rat hepatocytes (Massaad-Massade *et al.* 1998).

In the present study, we report that TNF α inhibits GST α expression and antagonizes the stimulatory actions of both FSH and testosterone on this enzyme mRNA and protein levels in cultured Sertoli cells. The inhibitory effect of TNF α on the basal GST α mRNA levels appears to result from a (negative) transcriptional activity since it did not affect GST α mRNA stability and since it was abolished by an inhibitor of transcription, DRB. However, further studies based, for example, on pulse-chase or the nuclear run-on assays, need to be performed to confirm the direct transcriptional regulatory action by TNF α on the *gsta* gene. On the other hand, the antagonistic interaction between TNF α and the two hormones occurs at distinct levels. Specifically, TNF α antagonizes FSH action by antagonizing the stimulatory effect of FSH on GST α mRNA stability. In contrast, the antagonistic interaction between TNF α and testosterone probably occurs at a transcriptional level as both testosterone and the cytokine did not directly affect GST α mRNA stability. Although it remains to be demonstrated that the inhibitory effect of TNF α on GST α expression in Sertoli cells reported here is also present *in vivo*, these observations indicate that the increase in the cytokine levels in the testis could affect the detoxification processes against the genotoxic products. Furthermore, in addition to the role in

Figure 6 Effect of TNF α on the FSH-stimulatory effect on GST α mRNA stability. Sertoli cells were incubated (A) in the presence (●) or absence (○) of TNF α and (B) in the presence of DHT (□) or DHT and TNF α (■). DRB (25 μ M) was then added to control and treated cells. GST α mRNA levels were evaluated at 0, 6, 12, and 24 h. Total RNA (10 μ g) from each time-point was analyzed by Northern blot with radiolabeled GST α cDNA probe. The data are plotted as the percentage of GST α mRNA remaining relative to that present at time zero. A representative pattern of three separate experiments is shown.

phase II detoxification, enzymes involved in the conjugation of electrophilic xenobiotics such as carcinogens and mutagens to the endogenous nucleophile GSH, GST α also contributes, particularly in the testis, to a major portion of the selenium-independent glutathione peroxidase activity toward phosphatidylcholine hydroperoxide (Hayes & Mantle 1986, Pickett & Lu 1989, Ketterer *et al.* 1990, Vos & Van Bladeren 1990). GST α may therefore be necessary to protect this tissue from reactive oxygen species-induced damage. The importance of GSTs in the protection against oxidative stress in testes is underscored by recent studies showing that, when GST activity is inhibited, accumulation of products of lipid peroxidation is augmented, resulting in germ cell apoptosis (Rao & Shaha 2000). More recently, these observations were further strengthened by data demonstrating that overexpression of GSTA2-2 (a member of the GST α class) in K562 cells attenuates the cytotoxic effect of H₂O₂ and other oxidants and protects against H₂O₂-induced apoptosis by blocking caspase 3 activation (Yang *et al.* 2001).

It has been reported that TNF α could exert a deleterious effect on male gonad activity, specifically on spermatogenesis. Indeed, (i) a severe hypogonadism was observed in male rats injected i.p. (Tulassay *et al.* 1970) or i.v.

(Christeff *et al.* 1987) with endotoxin, a substance known to stimulate TNF α production and (ii) recombinant human TNF α administration by continuous infusion to male rats induced severe seminiferous epithelium damage (Mealy *et al.* 1990). Although it has been shown that the disruption of the spermatogenic process in testes exposed to increased levels of TNF α could be related to a decrease in testosterone levels (Li *et al.* 1995, 1997, Mauduit *et al.* 1991, 1998), our present findings support the possibility that this spermatogenic arrest could also result from defects in detoxification processes in the gonad.

In summary, by using a model of cultured Sertoli cells, we report here that TNF α is a potent inhibitor of both the basal and hormone (FSH and androgen)-stimulated Sertoli cell GST α expression in terms of mRNA and proteins. Such an effect of the cytokine may alter the detoxification processes against genotoxic products in the testis.

Acknowledgements

This work was supported by Institut National de la Santé et de la Recherche Médicale (INSERM U 407) and the University of Lyon 1.

References

- Aceto A, Di Ilio C, Angelucci S, Felaco M & Federici G 1989 Glutathione transferase isoenzymes from human testis. *Biochemical Pharmacology* **38** 3653–3660.
- Benahmed M 1996 Growth factors and cytokines in the testis. In *Male Infertility*, pp 55–97. Ed. FH Comhaire. London: Chapman & Hall.
- Benbrahim-Tallaa L, Tabone E, Tosser-Klopp G, Hatey F & Benahmed M 2002 Glutathione S transferase alpha expressed in porcine Sertoli cells is under the control of follicle stimulating hormone and testosterone. *Biology of Reproduction* **66** 1734–1742.
- Boussouar F, Grataroli R, Jingwei J & Benahmed M 1999 Tumor necrosis factor- α stimulates lactate dehydrogenase A expression in porcine cultured Sertoli cells: mechanism of action. *Endocrinology* **140** 3054–3062.
- Bradford MM 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72** 248–254.
- Chomczynsky P & Sacchi N 1987 Single-step RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Analytical Biochemistry* **162** 156–159.
- Christeff N, Auclair M & Benassayag C 1987 Endotoxin-induced changes in sex steroid levels in male rats. *Journal of Steroid Biochemistry* **26** 67–71.
- De SK, Chen HL, Pace JL, Hunt JS, Terranova PF & Enders GC 1993 Expression of tumor necrosis factor- α in mouse spermatogenic cells. *Endocrinology* **133** 389–395.
- De Cesaris P, Starace D, Riccioli A, Padula F, Filippini A & Ziparo E 1998 Tumor necrosis factor α induces interleukin-6 production and integrin ligand expression by distinct transduction pathways. *Journal of Biological Chemistry* **273** 7566–7571.
- Friling RS, Bensimon A, Tichauer Y & Daniel V 1990 Xenobiotic-inducible expression of murine glutathione S-transferase Ya subunit gene is controlled by an electrophilic-responsive element. *PNAS* **87** 6258–6262.
- Gandy J, Primiano T, Novak RF, Kelce WR & York JL 1996 Differential expression of glutathione S-transferase isoforms in compartments of the testis and segments of the epididymis of the rat. *Drug Metabolism and Disposition* **24** 725–733.
- Gnessi L, Fabbri A & Spera G 1997 Gonadal peptides as mediators of development and functional control of the testis: an integrated system with hormones and local environment. *Endocrine Reviews* **18** 541–609.
- Grataroli R, Boussouar F & Benahmed M 2000 Role of sphingosine in the tumor necrosis factor α stimulatory effect on lactate dehydrogenase A expression and activity in porcine Sertoli cells. *Biology of Reproduction* **63** 1473–1481.
- Griswold MD 1995 Interactions between germ cells and Sertoli cells in the testis. *Biology of Reproduction* **52** 211–216.
- Hays JD & Mantle TJ 1986 Use of immuno-blot techniques to discriminate between the glutathione S-transferase Yf, Yk, Ya, Yn/Yb and Yc subunits and to study their distribution in extrahepatic tissues. *Biochemical Journal* **233** 779–788.
- Hays JD & Pulford DJ 1995 The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Critical Reviews in Biochemistry and Molecular Biology* **30** 445–600.
- Hays JD & Strange RC 1995 Invited commentary. Potential contribution of the glutathione S-transferase supergene family to resistance to oxidative stress. *Free Radical Research* **22** 193–207.
- Huston JC 1998 Interactions between testicular macrophages and Leydig cells. *Journal of Andrology* **19** 394–398.
- Ketterer B, Fraser G & Meyer DJ 1990 Nuclear glutathione transferases which detoxify irradiated DNA. *Advances in Experimental Medicine and Biology* **264** 301–310.
- Li X, Youngblood GL, Payne AH & Hales DB 1995 Tumor necrosis factor- α inhibition of 17 α -hydroxylase/C17–20 lyase gene (Cyp17) expression. *Endocrinology* **136** 3519–3526.
- Li X, Hales KH, Watanabe G, Lee RG, Pestell RG & Hales DB 1997 The effect of tumor necrosis factor- α and cAMP on induction of AP-1 activity in MA-10 tumor Leydig cells. *Endocrine* **6** 317–324.
- Liang Q, He JS & Fulco AJ 1995 The role of Barbie box sequences as cis-acting elements involved in the barbiturate-mediated induction of cytochromes P450_{BM-1} and P450_{BM-3} in *Bacillus megaterium*. *Journal of Biological Chemistry* **270** 4438–4450.
- Listowsky I, Rowe JD, Patskovsky YV, Tchaikovskaya T, Shintani N, Novikova E & Nieves E 1998 Human testicular glutathione S-transferases: insights into tissue-specific expression of the diverse subunit classes. *Chemico-Biological Interactions* **111** 103–112.
- Mannervick B & Danielson UH 1988 Glutathione S-transferase: structure and catalytic activity. *Critical Reviews in Biochemistry* **23** 283–337.
- Massa-Massade L, Chouaib S & Gouyette A 1998 Phenobarbital prevents the inhibitory effect of tumor necrosis factor on glutathione S-transferase mu in primary culture rat hepatocytes. *Anticancer Research* **18** 1833–1838.
- Mather JP & Philips DM 1984 Primary cultures of testicular somatic cells. In *Methods for Serum-Free Culture of Cells of the Endocrine System*, pp 29–45. Eds DW Barnes, DA Sirbasku & JH Sato. New York: Alan R. Liss.
- Mauduit C, Gasnier F, Rey C, Chauvin MA, Stocco DM, Louisot P & Benahmed M 1998 Tumor necrosis factor- α inhibits Leydig cell steroidogenesis through a decrease in steroidogenic acute regulatory protein expression. *Endocrinology* **139** 2863–2868.
- Mealy K, Robinson B, Millette CF, Majzoub J & Wilmore DW 1990 The testicular effects of tumor necrosis factor. *Annals of Surgery* **211** 470–475.
- Mehta R, David HG & Laver GW 1994 Glutathione S-transferases and P-glycoprotein in normal rat hepatocytes and hepatoma cells: analysis using flow cytometry. *Cancer Letters* **84** 163–172.
- Mueller A, Hermo L & Robaire B 1998 The effects of aging on the expression of glutathione S-transferases in the testis and epididymis of the Brown Norway rat. *Journal of Andrology* **19** 450–465.
- Murray GI, Taylor VE & McKay JA 1995 The immunohistochemical localization of drug metabolizing enzymes in prostate cancer. *Journal of Pathology* **177** 147–152.
- Papp S, Robaire B & Hermo L 1995 Immunocytochemical localization of the Ya, Yc, Yb1, and Yb2 subunits of glutathione S-transferases in the testis and epididymis of adult rats. *Microscopy Research and Technique* **30** 1–23.
- Pickett CB & Lu AYH 1989 Glutathione S-transferases: gene structure, regulation, and biological function. *Annual Review of Biochemistry* **58** 743–764.
- Rao AV & Shaha C 2000 Role of glutathione S-transferases in oxidative stress-induced male germ cell apoptosis. *Free Radical Biology and Medicine* **29** 1015–1027.
- Riccioli A, Filippini A, De Cesaris P, Barbacci E, Stefanini M, Starace G & Ziparo E 1995 Inflammatory mediators increase surface expression of integrin ligands, adhesion to lymphocytes, and secretion of interleukin 6 in mouse Sertoli cells. *PNAS* **92** 5808–5812.
- Rushmore TH, King RG, Paulson KE & Pickett CB 1990 Regulation of glutathione S-transferase Ya subunit gene expression: identification of a unique xenobiotic-responsive element controlling inducible expression by planar aromatic compounds. *PNAS* **87** 3826–3830.
- Rushmore TH, Morton MR & Pickett CB 1991 The antioxidant responsive element. *Journal of Biological Chemistry* **266** 11632–11639.
- Rushmore TH, Nguyen T & Pickett CB 1993 ARE and XRE mediated induction of glutathione S-transferase Ya subunit gene. In *Structure and Function of Glutathione S-Transferase*, pp 119–128. Eds KD Tew, CB Pickett, TJ Mantle, B Mannervick & JD Hays. Boca Raton, FL: CRC Press.
- Salinas AE & Wong MG 1999 Glutathione S-transferases – a review. *Current Medicinal Chemistry* **6** 279–309.

- Sawinski P, Lukaszyk A & Stolzmann WM 1996 An *in vivo* and *in vitro* study on the role of macrophages in local regulation of the Leydig cell function in the rat. *Folia Histochemica et Cytobiologica* **34** (Suppl 1) 81–82.
- Seidegard J & Ekstrom G 1997 The role of human glutathione transferases and epoxide hydrolases in the metabolism of xenobiotics. *Environmental Health Perspectives* **105** (Suppl 4) 791–799.
- Sundberg AG, Nilsson R, Appelkvist EL & Dallner G 1993 Localization of alpha and pi class glutathione transferases in normal human tissues. *Pharmacology and Toxicology* **72** 321–331.
- Tu CPD, Weiss MJ, Li NQ & Reddy CC 1983 Tissue-specific expression of the rat glutathione S-transferases. *Journal of Biological Chemistry* **258** 4659–4662.
- Tulassay Z, Viczian M, Bojthe L & Czeizel A 1970 Quantitative histological studies on the injury of spermatogenesis induced by endotoxin in rats. *Journal of Reproduction and Fertility* **22** 161–164.
- Vilcek J & Lee TA 1991 Tumor necrosis factor. *Journal of Biological Chemistry* **266** 7313–7316.
- Vos RM & Van Bladeren PJ 1990 Glutathione S-transferases in relation to their role in the biotransformation of xenobiotics. *Chemico-Biological Interactions* **75** 241–265.
- Yang Y, Cheng JZ, Singhal SS, Saini M, Pandya U, Awasthi S & Awasthi YC 2001 Role of glutathione S-transferases in protection against lipid peroxidation. Overexpression of hGST α 2-2 in k562 cells protects against hydrogen peroxide-induced apoptosis and inhibits JNK and caspase 3 activation. *Journal of Biological Chemistry* **276** 19220–19230.
- Yoganathan T, Eskild W & Hansson V 1989 Investigation of detoxification capacity of rat testicular germ cells and Sertoli cells. *Free Radical Biology and Medicine* **7** 355–359.

Received 24 May 2002

Accepted 19 August 2002