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Tumor necrosis factor-alpha mRNA stability in human peripheral blood cells after lipopolysaccharide stimulation

Abstract

TNF synthesis depends on many controls at transcriptional and post-transcriptional levels, including in particular mRNA stability and translational efficiency through the AU-rich elements (ARE) in the 3'untranslated region (3'UTR) of mRNA. We have previously reported that upon lipopolysaccharide (LPS) stimulation, TNF protein secreted by normal peripheral blood cells (PBC)

from non-Hodgkin's lymphoma patients was slightly, but not significantly increased when compared to healthy control donors. In contrast, the relative amounts of TNF mRNA were significantly higher in lymphoma patients. Thus, the implication of TNF mRNA stability has been explored by investigating the decay rate of LPS-induced TNF mRNA and the expression of tristetraprolin (TTP), one of the factors involved in the destabilization of TNF mRNA. After LPS incubation, peak levels of TTP mRNA preceded those of TNF mRNA, supporting its implication in the control of TNF mRNA levels in human PBC. Furthermore, similar TTP expression in both groups correlated with an identical decay rate of TNF mRNA, which excludes this pathway for the higher LPS-induced TNF mRNA levels in PBC from lymphoma patients.

Full text

INTRODUCTION

Considering the pleiotropism of tumor necrosis factor (TNF)-alpha, its biosynthesis is under the control of multiple and complex regulatory mechanisms at the transcriptional and post-transcriptional levels, involving the TNF gene promoter region and the AU-rich elements (ARE) present in the 3'untranslated region (3'UTR) of TNF mRNA [1]. Different protein complexes interfere with this latter region, modulating the stability and translational efficiency of TNF mRNA. Tristetraprolin (TTP), the prototype of a family of Cys-Cys-Cys-His zinc finger proteins, is one of these proteins, and is involved in the deadenylation and destabilization of TNF mRNA, leading to its degradation [2, 3]. Mice deficient for TTP develop an inflammatory syndrome mediated by excessive TNF production [4], implicating TTP in the regulation of TNF synthesis in mouse macrophages [5]. In the normal mouse macrophage, TTP biosynthesis is induced by the same agents that stimulate TNF production, including the lipopolysaccharide (LPS) and TNF itself [3]. Then, TNF may participate in an autoregulatory negative feed back loop.

Since elevated levels of circulating TNF have been associated with patient outcome in non-Hodgkin's lymphoma [6, 7], we further investigated the production of TNF protein and mRNA in peripheral blood cells (PBC) from these patients. We have previously reported that upon LPS stimulation, TNF protein secreted by PBC from non-Hodgkin's lymphoma patients was slightly but not significantly increased when compared to healthy control donors. In contrast,

the relative amounts of TNF mRNA were significantly higher in lymphoma patients [8]. This TNF mRNA increase (about 2 fold) was strongly related to enhanced gene transcription. Moreover, the lack of a direct relationship between TNF transcript and secreted TNF protein observed in these PBC, suggested that additional mechanisms at the translational or post-translational levels may impair TNF protein production in patient's cells [8] and possibly the stability of TNF mRNA. In order to confirm these earlier observations, the decay rate of LPS-induced TNF mRNA was determined. In addition, TTP mRNA expression was evaluated in the same whole blood model and in the human monocytic cell line THP-1, as a control of monocytic response.

MATERIALS AND METHODS

Subjects

Twenty-one, newly diagnosed, consecutive lymphoma patients (all lymphoma types) and ten healthy subjects as controls were included in this study, after having given their informed consent [8]. The patients had not received any previous treatment. Patients in which lymphoma cells could be detected in peripheral blood by cytological analysis or flow cytometry were not eligible for this study, in order to avoid the possibility of stimulating circulating lymphoma cells.

Whole blood cell culture

The blood samples were collected in apyrogenic heparinized tubes (Endo Tube-ET, Chromogenix AB, Sweden) and immediately processed as originally described [9], and previously used for TNF mRNA expression [8]. Briefly, blood was diluted ten fold in RPMI 1640 (Eurobio, Les Ulis, France) complemented with 2 mM glutamine (Gibco-BRL, Grand Island, NY, USA), 100 U/ml penicillin, 100 mug/ml streptomycin (final concentrations) and was then distributed into 1-ml wells. The mononuclear cells were stimulated with 1 mug/ml of LPS (*Escherichia coli* serotype 055:B5, Sigma, St. Louis, MO, USA). Plates were incubated at 37° C under 5% CO_2 for 30 min, 2, 4, 8 and 24 hours respectively. For each incubation time, the contents of 3 wells (about 10^6 cells) were collected and centrifuged at 1,500 rpm for 10 min at 4° C. The cellular pellets were then harvested for RNA extraction. In addition, a range of LPS concentrations (0.1, 1 and 10 ng/ml) was tested for the first incubation time. In some experiments, the transcription

inhibitor actinomycin D (Sigma) was added (5 mug/ml) to the culture for 15 min before LPS stimulation.

Half-life of TNF mRNA

For TNF mRNA stability experiments, diluted-whole blood was stimulated with 1 mug/ml of LPS for 1 hour (37° C under 5% CO_2), and actinomycin D (5 mug/ml) was added to the culture medium for 1 hour in order to inhibit further transcription. Cells were harvested for the extraction of RNA at 15-min intervals, for 1 hour.

Cell line

The human monocytic cell line THP-1 (DSMZ, Braunschweig, Germany) was maintained in RPMI 1640 medium (Eurobio) supplemented with 2 mM glutamine (Gibco-BRL), 100 U/ml penicillin, 100 mug/ml streptomycin (final concentrations) and 10% fetal bovine serum (Vysis, Voisins-le-Bretonneux, France). For the cell stimulation, 5×10^5 cells per ml were further incubated with or without LPS for the indicated periods, in fresh, complemented RPMI 1640. The transcription inhibition by actinomycin D has been studied in THP-1 as previously described for a whole blood model. The cell viability as determined by the trypan blue exclusion test was > 95% before stimulation and for each culture period.

RNA isolation and cDNA synthesis

After 2 washes in phosphate-buffered saline, RNA was extracted from the cells using the TRIZOL reagent (Gibco-BRL) and dissolved in 10 mul of diethylpyrocarbonate-treated distilled water. In the first step, 5 mul of the total RNA solution was incubated with 10 pmol of oligo-dT at 65° C for 8 min. Then, cDNA synthesis was performed in a total volume of 20 mul, consisting of 15 U/mul of M-MLV reverse transcriptase and the recommended buffer (Gibco-BRL), 500 muM each of dNTP (Gibco-BRL), 10 mM dithiothreitol and 2 U/mul of RNasin (Promega, Madison, WI, USA). The reaction mixture was incubated in a water bath at 37° C for 90 min, followed by 3 min of heat inactivation at 95° C.

Quantification of TNF and TTP transcripts

The TNF and TTP transcripts were quantified by real-time PCR [10] using the ABI Prism® 7700 Sequence Detector System (Perkin Elmer/Applied Biosystem, Foster City, CA, USA), as previously described for quantification of TNF transcripts [8]. In order to normalize any possible variations in mRNA extraction and cDNA synthesis, expression of the porphobilinogen deaminase (PBGD) [11] housekeeping gene was also measured. The primers and TaqMan probes were designed for the amplification of the different cDNA using the Primer Express Software program (version 1.0 ppd; Perkin Elmer/Applied Biosystem) (Table 1). The cDNA of the target TTP and reference PBGD were amplified in separate tubes. Each reaction was performed using 2 mul of the cDNA in a total volume of 25 mul with 4 mM $MgCl_2$, 200 muM dATP, dGTP, dCTP and 400 muM dUTP, 1 X TaqMan buffer A, 0.01 U/mul uracil-N-glycosylase, 0.025 U/muL AmpliTaq Gold polymerase (Perkin Elmer/Applied Biosystem), and for TTP amplification: 100 nM of primers, 200 nM of TTP probe or for PBGD amplification: 200 nM of primers, 200 nM of the PBGD probe. Both reactions comprised 10 min at 95° C, then 40 cycles with 15 sec at 95° C and 60 sec at 60° C. All samples were tested in duplicate at selected times and the threshold cycles (Ct) were averaged from the values obtained in each reaction. In all experiments, the variation of duplicate Ct values did not exceed 2%. Quantification of the TTP transcript was performed using the comparative Ct method, which allows a relative quantification of gene expression where the target gene Ct is compared with the reference gene Ct, as demonstrated [12].

Statistical analysis

Comparisons of TTP mRNA values between the patients and controls were performed using the Mann-Whitney U-Test. The correlation between the different data was determined using the Spearman rank test. A p value of less than 0.05 was considered to be significant.

RESULTS

Relative TNF mRNA quantification in lymphoma patient cells and controls: kinetics and effects of LPS

At each incubation time, the amounts of TNF mRNA were normalized to an endogenous referred PBGD and referred to the t = 0 mRNA level as a calibrator. Upon LPS stimulation, the relative amounts of TNF increased quickly, with the

maximum level observed at 2 and 4 hours, which then decreased until 24 hours. At each incubation time, the median of relative TNF mRNA levels was 1.5 to 2 fold higher in lymphoma patients than in controls (Figure 1). The differences observed between lymphoma patients and healthy subjects were statistically significant at 30 min, 4 and 24 hours ($p < .05$). To determine whether this increase of TNF mRNA levels resulted from a modified transcriptional activity, the PBC were preincubated with actinomycin D for 15 min and then stimulated with LPS for 30 min. In the presence of actinomycin D, a sharp decrease in LPS-induced TNF mRNA levels was observed, of 90% for lymphoma patients, and of 86% for healthy subjects, whereas the PBGD mRNA levels at 30 min were not affected by this transcription inhibitor (data not shown). These results suggest that the increase in TNF mRNA levels at t = 30 min in both groups was due to enhanced gene transcription rather than to an accumulation of TNF mRNA. Thus, the relative mRNA levels measured 30 min after LPS stimulation are likely to correspond to newly synthesized mRNA and may therefore represent LPS-induced transcriptional activity of the TNF gene.

In order to verify that the 2-fold higher LPS-induced TNF mRNA was not dependent on the LPS concentration (1 mug/ml) used in the experimental model, the levels of TNF mRNA were determined at several LPS doses (0.1; 1; 10 and 1,000 ng/ml). After incubation with LPS, the relative amounts of TNF mRNA increased rapidly. At each LPS concentration, the median of relative TNF mRNA levels was 1.6 to 2 fold higher in lymphoma patients than in controls (Figure 2), suggesting that the difference observed between both groups was independent of the LPS concentration.

TNF mRNA stability

Thus the LPS-stimulated TNF mRNA levels, which were independent of the LPS concentration used, were consistently higher in PBC from lymphoma patients and were probably related to enhanced gene transcription. Neverthless, this difference might also result from modification of TNF mRNA stability. In order to rule out this latter hypothesis, the half-life of TNF mRNA was measured in whole blood cultures. After incubation of PBC with LPS for 1 hour, followed by addition of actinomycin D, a slow decline of the cellular TNF mRNA content over 15 to 60 min was observed in both groups (Figure 3). No significant differences were observed between lymphoma patients and controls (estimated TNF mRNA half-life 39.0 min and 34.5 min, respectively), indicating that the differences in TNF

mRNA levels in the present system are independent of mRNA stability. This observation emphasizes the importance of transcriptional activity in the increased levels of TNF mRNA.

Quantification of TTP mRNA

Because TTP has been identified as a protein that promotes the deadenylation and the destabilization of TNF mRNA, and since these mechanisms are associated with the initiation of mRNA degradation, the TTP mRNA levels were quantified in whole blood cultures. Real time PCR was used to determine the level of TTP mRNA at selected times. The amount of TTP mRNA at each incubation time, with or without LPS stimulation, was normalized to an endogenous reference PBGD, and referred to the t = 0 mRNA level as a calibrator. In unstimulated cultures, normal PBC contained a low but detectable relative level of TTP mRNA (Table 2, upper panel). After LPS stimulation, this level increased, quickly and significantly, with the maximum level observed at 30 min. Then, it declined until 4 hours and remained stable until 24 hours (Table 2, lower panel) (Figure 4A). Identical LPS-induced TTP mRNA kinetics were observed with the human monocytic cell line THP-1 (Figure 4B). In a previous study [8], LPS stimulation increased the relative amounts of TNF mRNA in PBC significantly. Nevertheless, the TNF mRNA production occurred less rapidly than TTP mRNA, showing a peak at 2 and 4 hours (Figure 4A). Thus, in response to LPS, the peak of TTP mRNA production preceded the maximal level of TNF mRNA, suggesting the possible control by TTP of cellular levels of TNF mRNA Furthermore, at each incubation time, no statistical differences were observed between the median of relative LPS-induced TTP mRNA from lymphoma patients and from healthy subjects.

Inhibition by actinomycin D of TTP gene transcription after LPS stimulation

In order to assess whether the amounts of quantified TTP mRNA, at least at 30 min, resulted from transcription, PBC from lymphoma patients and from healthy subjects were preincubated with actinomycin D for 15 min, and were then stimulated with LPS for 30 min (Figure 5). These results suggest that the increase in TTP mRNA levels at t = 30 min in both groups, resulted from enhanced gene transcription rather than an accumulation of TTP mRNA. The same transcription inhibition at 30 min (96%) was observed in THP-1 (data not shown).

DISCUSSION

This study describes the decay rate of LPS-induced TNF mRNA, which is similar in PBC from lymphoma patients and controls, and its relation with TTP expression.

The identical mRNA stability in both groups suggests that the enhanced TNF mRNA levels in LPS-stimulated PBC from lymphoma patients do not result from mRNA accumulation, but from the increased transcriptional activity in these cells [8]. Since TTP was implicated in the destabilizing of TNF mRNA in mouse macrophages [2], the LPS-induced TTP mRNA expression was therefore determined. The peaks of TTP mRNA (t = 30 min) probably resulted from newly transcribed mRNA, as indicated by the experiments using actinomycin D, and may therefore represent the LPS-induced transcriptional activity of the TTP gene. So, in human PBC, LPS stimulation may induce the transcription of the TTP gene. These results are consistent with the first description of LPS-induced TTP mRNA expression in mouse models [3]. The conservation of LPS-induced TTP expression in mice and humans emphasizes its critical role in the regulatory mechanism of TNF synthesis. *In vivo*, in both mice and in human cells, upon LPS stimulation, TTP mRNA levels increase rapidly (*e.g.* 30 min), preceding those of TNF (*e.g.* 2 hours). The kinetics of TTP and TNF mRNA are thus compatible with the fact that TTP may partially control the cellular levels of TNF mRNA, inducing the destabilization already demonstrated in mouse cells. The identical LPS-induced TTP expression in PBC from lymphoma patients and from controls may explain the similar half-life of TNF in both groups. However, if TTP has been associated with negative regulation of TNF mRNA stability, other factors as HuR/ELAV family proteins, binding ARE, may also mediate the stabilization of mRNA [13]. Thus, the determination of mRNA stability by ARE appears complex, involving negative-positive regulatory loops. Furthermore, eukaryotic mRNA can be simultaneously degraded by several mechanisms (mainly the deadenylation-dependent pathway), resulting in mRNA levels that will be the summation of decay rates through each different pathway [14]. In the present model, no significant correlation was found between the peaks for TTP mRNA and TNF mRNA (data not shown). Since TTP protein is subject to many modes of regulation itself [15], further studies may confirm its direct link with TNF mRNA in this model.

On the other hand, LPS-induced TNF mRNA stability does not seem implicated in the difference in TNF protein concentrations between the both groups, indicating that other pathways controlling TNF synthesis may be modified. Firstly, translational activation is one of the important mechanisms regulating TNF synthesis that accounts for the rapid induction of TNF protein production following stimulation by an appropriate inducer such as LPS [16]. The ARE in the 3'UTR of TNF mRNA, described as controlling mRNA stability, can independently exert a role in translation efficiency [17, 18]. Indeed, a protein such as TIA-1 appears to function as a translational silencer in the mouse macrophage [19]. The translation rate and/or its efficiency may therefore be modified in PBC from lymphoma patients after LPS stimulation. Interestingly, interleukin-10 suppresses TNF expression, mainly by targeting the ARE of TNF mRNA, and by modulating the translation independently of TTP [20]. Finally, other steps in the secretory pathway may also be involved, such as cleavage of TNF precursor to yield the mature, secreted product [21, 22].

CONCLUSION

In conclusion, these experiments have allowed us to describe, in human PBC, the kinetics of TTP mRNA in response to LPS stimulation, a potential target for the regulation of inappropriate TNF production observed in several clinical situations. The similar decay rate of TNF mRNA seen in cells from lymphoma patients and controls, confirms that the increased TNF mRNA levels observed in these cells result from a higher transcriptional activity. This study underlines the fact that appropriate TNF biosynthesis requires many controls at the transcriptional and post-trancriptional levels, resulting in a complex network of positive and negative regulation pathways implicating TNF itself.

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Figures

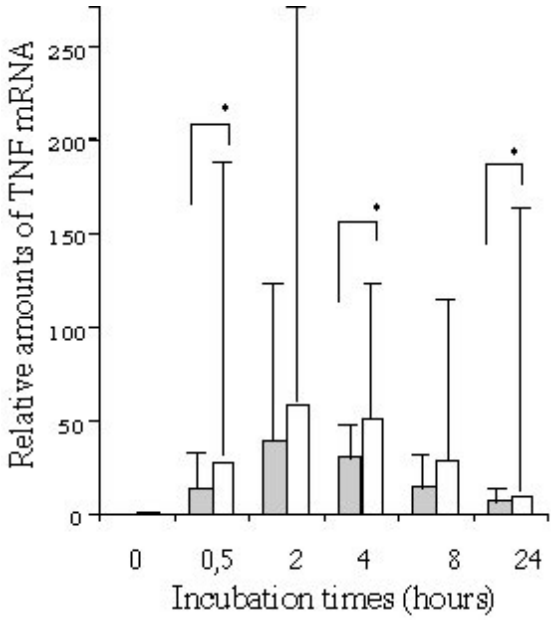


Figure 1
Relative TNF mRNA levels after LPS stimulation. The levels of TNF mRNA at each incubation time were

normalized to an endogenous reference PBGD and referred respectively to t = 0 TNF mRNA level as calibrator. Results are expressed as median for controls (grey bars, n = 10), and for lymphoma patients (open bars, n = 21), with error bars as extreme upper values. * indicates significant ($p < .05$) differences between lymphoma patients and controls.

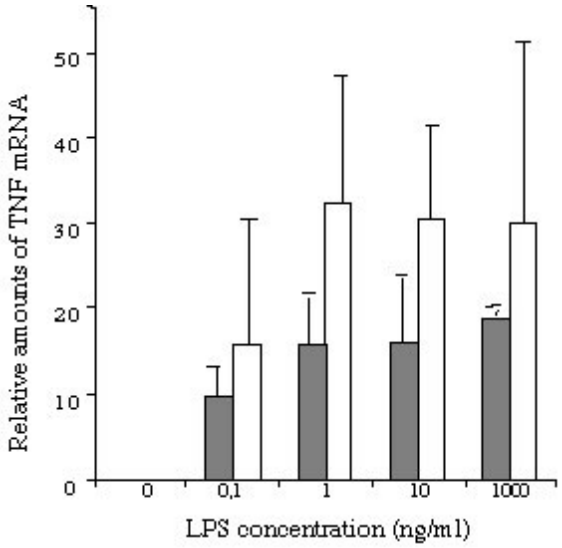


Figure 2
Relative TNF mRNA levels according to LPS concentration. After LPS incubation (t = 30 min), the levels of TNF mRNA at each LPS concentration were normalized to an endogenous reference PBGD and referred respectively to t = 0 TNF mRNA level as calibrator. Results are expressed as median for controls (grey bars, n = 5), and for lymphoma patients (open bars, n = 5) with error bars as extreme upper values.

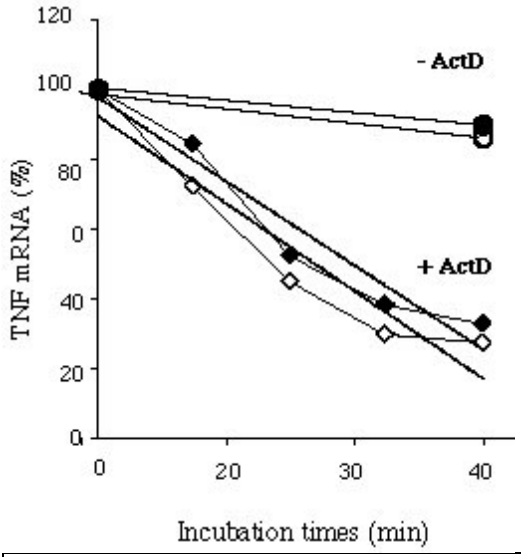
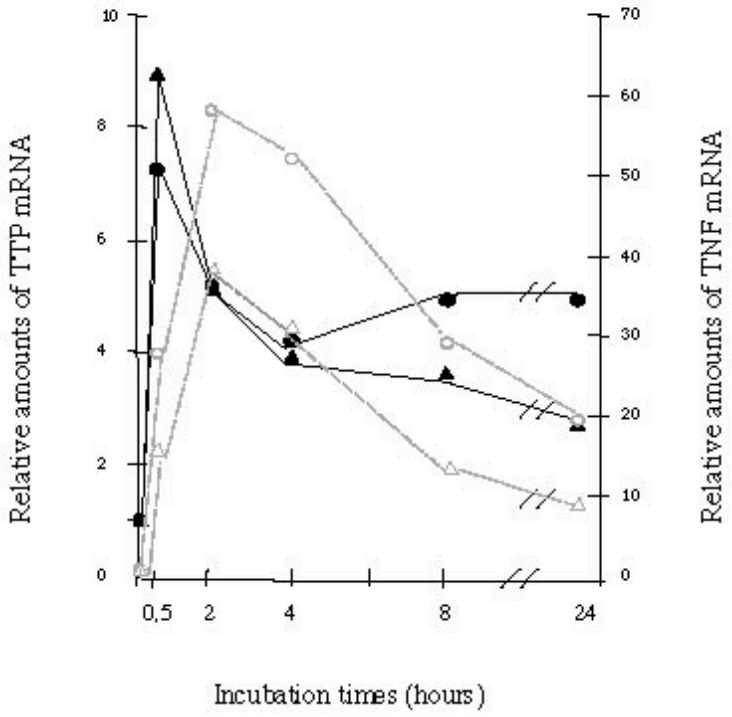


Figure 3

Stability of TNF mRNA. The amount of relative TNF mRNA at time 0 of actinomycin D (actD) treatment was taken as 100%. Results are expressed as median for lymphoma patients (closed diamond - with actD treatment -, closed circle - without actD treatment -, n = 5), and for controls (open diamond - with actD treatment - open circle - without actD treatment -, n = 5). The dotted lines represent the linear regression of TNF mRNA decay. Using these linear regressions, the estimated half-lives TNF mRNA were found to be 39.5 min in patients and 34.0 min in controls.

A/



B/

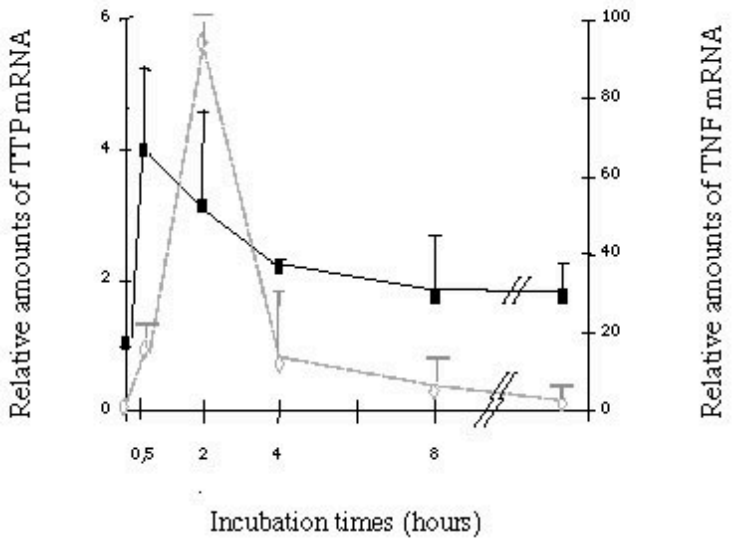


Figure 4

Kinetics of relative TTP and TNF mRNA after LPS stimulation. The amounts of TTP and TNF mRNA at each time point were normalized to an endogenous reference PBGD and referred respectively to t = 0 TTP and TNF mRNA level as calibrator. **A:** Results are expressed as median (TTP mRNA = closed circle in lymphoma patient

cells and closed triangle in control cells; TNF mRNA = open circle and open triangle in lymphoma patient and control cells respectively). **B:** Results are expressed as the mean of 2 independent experiments (± SD) for the cell line THP-1 (TTP mRNA = closed square, TNF mRNA = open diamond).

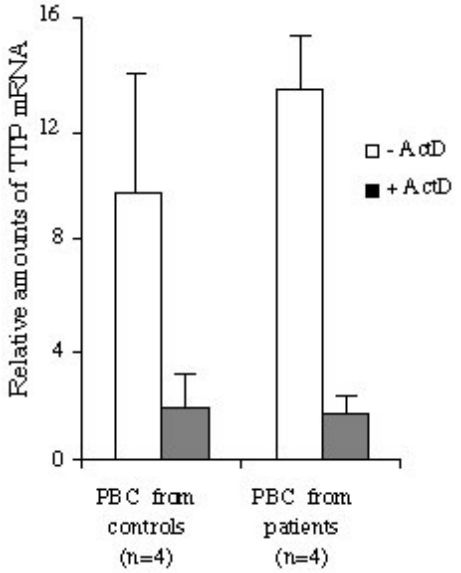


Figure 5

Transcription inhibition of the TTP gene by actinomycin D. Results are expressed as the median of relative TTP mRNA levels obtained for PBC from 4 lymphoma patients and 4 controls at t = 30 min. Without actinomycin D, the median of relative TTP mRNA levels in PBC from lymphoma patients was 13.4, as compared to 1.7 in the presence of actinomycin D, corresponding to a transcription inhibition of 88%. In healthy subjects, the median of relative TNF mRNA levels was 9.6 as compared to 1.8 in the presence of actinomycin D, corresponding to a transcription inhibition of 81%. The PBGD mRNA levels at 30 min were not affected by this transcription inhibitor (data not shown). Error bars represented extreme upper values.

Table 1
Primers and probe sequences
The primers and TaqMan™ probes for the amplification of TNF and PBGD cDNA have been described elsewhere [8]

Primers and probes	Sequence (5'→ 3')	Reporter dye in 5'	Exon	Annealing temperature (° C)
TTP forward	ATGGATCTGACTGCCATCTACGAG	–	1	60
TTP reverse	GCTGGACTCAGTCCCTCCATG	–	2	61
TTP probe	CTCCTGTCGCTGAGCCCT	Fam	2	68

Table 2
Relative amounts of TTP mRNA in unstimulated and stimulated conditions

		t = 0	t = 30 min	t = 2 hours	t = 4 hours	t = 8 hours	t = 24 hours
Unstimulated conditions	Controls (n = 10)	1.0	1.1 (0.4-3.4)	2.3 (0.7-4.1)	3.3 (0.9-5.6)	1.7 (0.7-5.8)	0.9 (0.6-3.6)
	Patients (n = 21)	1.0	1.1 (0.2-10.0)	1.6 (0.2-5.8)	2.4 (0.8-15.1)	1.9 (0.6-7.8)	1.6 (0.5-7.5)
Stimulated Conditions	Controls (n = 10)	1.0	7.2 (2.1-25.7)	4.7 (4.0-8.9)	3.1 (1.1-6.8)	3.0 (0.4-18.2)	2.8 (0.9-4.8)
	Patients (n = 21)	1.0	7.3 (4.2-93.7)	5.2 (1.2-30.4)	4.2 (1.5-13.5)	4.9 (0.6-37.8)	5.0 (1.2-17.1)

Results are presented as median, with ranges in parenthesis.

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IL-1alpha, IL-1beta, organ cytokine expression, young/old age, homeostasis.

The combination of the interleukin-1alpha (IL-1alpha-889) genotype and the interleukin-10 (IL-10 ATA) haplotype is associated with increased interleukin-10 (IL-10) plasma levels in healthy individuals

Sanna Kilpinen, Heini Huhtala, Mikko Hurme
interleukin-1, interleukin-10, gene polymorphism, plasma.

The interleukin-6 G(-174)C polymorphism and the ex vivo interleukin-6 response to endotoxin in severely injured blunt trauma patients

Michael Heesen, Udo Obertacke, F. Ulrich Schade, Brunhilde Bloemeke, Matthias Majetschak
interleukin-6, endotoxin, genetic polymorphism, polymerase chain reaction, whole blood, critical illness.

Comparison of leptin- and interleukin-6-regulated expression of the rPAP gene family: evidence for differential co-regulatory signals

Daniël Broekaert, Sven Eyckerman, Delphine Lavens, Annick Verhee, Wim Waelput, Joël Vandekerckhove, Jan Tavernier
forskolin, IL-6, leptin, rPAP/Reg gene family, signal transduction.

Mice lacking alpha2-macroglobulin show an increased host defense against Gram-negative bacterial sepsis, but are more susceptible to endotoxic shock

Tino Hochepied, Fred Van Leuven, Claude Libert
TNF, shock, acute phase, inflammation, alpha2-macroglobulin.

Tumor necrosis factor-alpha mRNA stability in human peripheral blood cells after lipopolysaccharide stimulation

Lucile Baseggio, Carole Charlot, Jacques Bienvenu, Pascale Felman, Gilles Salles
tumor necrosis factor, mRNA stability, lipopolysaccharide, tristetraprolin.

Caspase-1 levels in biological fluids from patients with multiple sclerosis and from patients with other neurological and non-neurological diseases

Diego Franciotta, Gianvito Martino, Elisabetta Zardini, Roberto Furlan, Roberto Bergamaschi, Maira Gironi, Alessandra Bergami, Giovanna Angelini, Fabrizio De Benedetti, Patrizia Pignatti, Gianna Moscato, Vittorio Cosi
asthma, cerebrospinal fluid, juvenile idiopathic arthritis, sputum, synovial fluid, THP-1 cells.

Divergent effects of tumor necrosis factor-alpha and lymphotoxin-alpha on lethal endotoxemia and infection

with live Salmonella typhimurium in mice

Edi Dharmana, Monique Keuter, Mihai G. Netea, Ineke C.M.M. Verschueren, Bart Jan Kullberg
tumor necrosis factor, lymphotoxin, salmonella typhimunium, knock-out mice.

Th1/Th2 cytokine balance and nitric oxide in cerebrospinal fluid and serum from patients with multiple sclerosis

María del Carmen Rodríguez-Sáinz, Silvia Sánchez-Ramón, Clara de Andrés, Margarita Rodríguez-Mahou, M Angeles Muñoz-Fernández
cytokine, nitric oxide, multiple sclerosis, cerebrospinal fluid, oligoclonal bands, Igs.

Retinoids, cisplatin and interferon-alpha in recurrent or metastatic cervical squamous cell carcinoma: clinical results of 2 phase II trials

Anne-Chantal Braud, Laurence Gonzague, François Bertucci, Dominique Genre, Jacques Camerlo, Gwenaelle Gravis, Anthony Goncalves, Vincent Moutardier, Frédéric Viret, Dominique Maraninchi, Patrice Viens
all-trans-retinoic acid, 13 Cis retinoic acid, interferon-alpha, cervical squamous cell carcinoma.

Circulating macrophage colony stimulating factor as a marker of tumour progression

Raymond S. McDermott, Laurent Deneux, Véronique Mosseri, Jacques Védrenne, Krishna Clough, Alain Fourquet, José Rodriguez, Jean-Marc Cosset, Xavier Sastre, Philippe Beuzeboc, Pierre Pouillart, Susy M. Scholl
CSF-1, tumour progression, metastasis, de-differentiation.

The effect of interleukin-1beta (IL-1beta) and tumor necrosis factor-alpha (TNF-alpha) on granulocyte macrophage-colony stimulating factor (GM-CSF) production by neuronal precursor cells

J. Benjamin Dame, Nasser Chegini, Robert D. Christensen, Sandra E. Juul
IL-1beta, TNF-alpha, GM-CSF, neuron, mRNA, protein.

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