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Inhibition of tristetraprolin expression by dexamethasone in activated macrophages

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Abstract

Tristetraprolin (TTP) is a factor that regulates mRNA stability and the expression of certain inflammatory genes. In the present study, we found that TTP expression was increased in macrophages exposed to bacterial lipopolysaccharide (LPS). Dexamethasone and dissociated steroid RU24858 inhibited LPS-induced TTP protein and mRNA expression and the inhibitory effect was reversed by a glucocorticoid receptor antagonist mifepristone. Histone deacetylase inhibitors trichostatin A (TSA) and apicidin reduced the inhibitory effect of dexamethasone and RU24858 on TTP expression, but the glucocorticoids did not alter TTP mRNA half-life. These results suggest that anti-inflammatory steroids reduce TTP expression in activated macrophages by a glucocorticoid response element (GRE)-independent mechanism, possibly through histone deacetylation and transcriptional silencing.

Keywords: Glucocorticoid; Histone deacetylation; Inflammation; Mitogen-activated protein kinase; mRNA stability; Tristetraprolin

1. Introduction

Tristetraprolin (TTP), also known as Nup475, TIS11, G0S24 and Zfp36, was first described as an immediate early response gene in cells stimulated by growth factors and mitogens and by factors like cycloheximide, forskolin and 12-*O*-tetradecanoylphorbol-13-acetate ester [1–6]. TTP belongs to a family of CCCH tandem zinc-finger proteins together with Zfp36l1 (Zfp36-like 1, also known as Berg36, BRF1, cMG1, ERF1, TIS11b) and Zfp36l2 (Zfp36-like 2, also known as BRF2, ERF2 and TIS11d) [7]. Although these proteins are differentially expressed, they have structural similarities: they all have two Cys-Cys-Cys-His zinc-finger domains, which have RNA-binding properties [8,9]. TTP mRNA is widely expressed at particularly high levels in spleen, lymph nodes and thymus [1,3].

TTP is involved in the regulation of inflammatory responses. A direct connection between TTP and inflammation was demonstrated in mice lacking the TTP gene [10]. TTP knock-out mice were found to develop a severe inflammatory syndrome, which was associated with elevated levels of circulating tumor necrosis factor α (TNF-α). Increased TNF-α production in these mice was found to be due to increased mRNA stability [11,12]. In subsequent studies, TTP has been shown to bind to the A + U-rich element in the TNF-α 3′-untranslated region which promotes deadenylation and destabilization of the TNF-α mRNA [13]. The mRNA of the granulocyte-macrophage colony-stimulating factor is also destabilized by TTP, which is assumed to be the reason for myeloid hyperplasia observed in TTP-deficient mice [14]. The stability of the mRNAs of interleukin-3 and cyclooxygenase-2 are also regulated by TTP [15,16].

TTP is known to be involved in the expression of certain inflammatory genes, but the regulation of the expression of TTP itself remains largely unknown. The aim of the

Abbreviations: Erk1/2, extracellular signal-regulated kinase 1/2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GR, glucocorticoid receptor; GRE, glucocorticoid response element; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κB; PD 98059, 2-(2′-amino-3′-methoxyphenyl)-oxanaphthalen-4-one; PDTC, pyrrolidinedithiocarbamate; SB 202474, 4-ethyl-2-(4-methoxyphenyl)-5-(4-pyridyl)-imidazole; SB 203580, 4-(4-fluorophenyl)-2-(4-methylsulphinylphenyl)-5-(4-pyridyl)-imidazole; SP 600125, anthra[1,9-*cd*]pyrazol-6(2*H*)-one; TNF-α, tumor necrosis factor α; TSA, trichostatin A; TTP, tristetraprolin

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present study was to investigate the regulation of TTP expression in macrophages exposed to an inflammatory stimulus [lipopolysaccharide (LPS)], and especially the effect of anti-inflammatory steroids.

2. Materials and methods

2.1. Materials

Reagents were purchased as follows: Tyrphostin AG-490, 2-(2′-amino-3′-methoxyphenyl)-oxanaphthalen-4-one (PD 98059), anthra[1,9-*cd*]pyrazol-6(2*H*)-one (SP 600125), cyclosporin A, 4-ethyl-2-(4-methoxyphenyl)-5-(4-pyridyl)-imidazole (SB 202474) and 4-(4-fluorophenyl)-2-(4-methylsulphinylphenyl)-5-(4-pyridyl)-imidazole (SB 203580) from Calbiochem, apicidin from Alexis Corporation, genistein from Tocris, dexamethasone from Orion Corp., and RU24858 was received from Aventis Pharma. All other reagents were from Sigma unless otherwise stated.

2.2. Cell culture

J774 murine macrophages (American Type Culture Collection) were cultured at 37 °C in humidified 5% carbon dioxide atmosphere in Dulbecco's modified Eagle medium with Ultraglutamine 1 (Cambrex Bioproducts Europe) supplemented with 10% heat-inactivated fetal bovine serum (Cambrex Bioproducts Europe), penicillin (100 units/ml), streptomycin (100 μg/ml) and amphotericin B (250 ng/ml) (Gibco) and harvested with trypsin–EDTA (Gibco). Cells were seeded on 6- or 24-well plates and grown to confluence prior to the experiments.

2.3. RNA extraction and quantitative real-time PCR

Cells were homogenized using QIAshredder™ (QIAGEN Inc.) after which total RNA was extracted with RNeasy® Mini kit (QIAGEN Inc.). The amount of RNA was measured with a spectrophotometer and the purity was confirmed by the absorbance ratio at $A_{260/280}$. Reverse transcription and quantitative PCR were performed according to the manufacturer's instructions (Applied Biosystems). Reverse transcription was carried out with TaqMan Reverse Transcription reagents and random hexamers in 10 μl reaction volume containing 25 ng purified RNA. Gene transcript levels of the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and mouse TTP were quantified by real-time PCR on ABI PRISM® 7000 Sequence Detection System (Applied Biosystems). Approximately 1 ng of total RNA reverse-transcribed into cDNA was used in the polymerase chain reaction applying TaqMan® Universal PCR Master Mix and sequence detection primers and TaqMan TAMRA™ probes. Rodent GAPDH Control Reagents were obtained from Applied Biosystems and primer and probe concentrations were optimized and primers and probes were used in the following concentrations: both forward and reverse rGAPDH primers 300 nM, rGAPDH probe 50 nM containing VIC™ (proprietary dye, Applied Biosystems) as 5′-reporter dye and TAMRA (6-carboxy-tetramethyl-rhodamine) as 3′-quencher. The mouse TTP primers and probe were designed using Primer Express® Software (Applied Biosystems) and were 5′-CTCAGAAAGCGGGCGTTGT-3′ (TTP forward, GenBank accession no. NM_011756, 343–361), 5′-GATTGGCTTGGCGAAGTTCA-3′ (TTP reverse, 400–419), 5′-CCAAGTGCCAGTTTGCTCACGGC-3′ [TTP probe containing 6-FAM (6-carboxy-fluoroscein) as 5′-reporter dye and TAMRA as 3′-quencher, 372–394] and used in concentrations of 300, 300 and 200 nM respectively (all from Metabion). Thermal cycling conditions were: incubation in 50 °C for 2 min, 95 °C for 10 min, thereafter 40 cycles of denaturation in 92 °C for 15 s and annealing/extension in 60 °C for 1 min. Each sample was determined in duplicate.

The relative mRNA levels were quantified and compared using the relative standard curve method as described in Applied Biosystems User Bulletin #2. Total RNA was isolated from LPS-stimulated J774 macrophages and reverse transcribed. Standard curves for GAPDH and TTP were created using dilution series of cDNA corresponding approximately 1 pg to 10 ng of total RNA in PCR as described above. The threshold cycle values obtained were plotted against dilution factor to create a standard curve. Relative mRNA levels in test samples were then calculated using the standard curve. The relative amount of gene transcript present was calculated and normalized by dividing the calculated value of TTP by the GAPDH value in each sample.

2.4. Western blotting

After the time indicated, J774 macrophages were washed with ice-cold PBS and lysed in ice-cold extraction buffer [10 mM Tris–base, 5 mM EDTA, 50 mM NaCl, 1% (w/v) Triton-X-100, 0.5 mM phenylmethylsulfonyl fluoride, 2 mM sodiumorthovanadate, 10 μg/ml leupeptin, 25 μg/ml aprotinin, 1.25 mM NaF, 1 mM sodium pyrophosphate, 10 mM *n*-octyl-β-D-glucopyranoside]. After a 15-min incubation on ice and centrifugation (13 400 × *g*, 4 °C, 10 min), supernatants were collected and stored in sample buffer [62.5 mM Tris–HCl, pH 6.8, 10% (v/v) glycerol, 2% (w/v) SDS, 0.025% (w/v) bromophenol blue, 5% (v/v) β-mercaptoethanol] at −20 °C. An aliquot of the supernatant was used to determine protein concentration by the Coomassie blue method [17]. Prior to Western blotting, proteins were boiled for 5 min with sample buffer [62.5 mM Tris–HCl, pH 6.8, 10% (v/v) glycerol, 2% (w/v) SDS, 0.025% (w/v) bromophenol blue, 5% (v/v) β-mercaptoethanol] and 20 μg of protein was used per lane on 12% SDS-polyacrylamide gel and transferred to Hybond

ECL™ nitrocellulose membrane (Amersham). After transfer, the membrane was blocked with TBS/T [20 mM Tris–base pH 7.6, 150 mM NaCl, 0.1% (v/v) Tween-20] containing 5% (w/v) bovine serum albumin. Thereafter the membrane was incubated with TTP antibody (Santa Cruz Biotechnology) in the blocking buffer for 1 h at room temperature. The membrane was washed and incubated with the secondary antibody in TBS/T containing 5% (w/v) milk powder (30 min at room temperature), and thereafter the bound antibody was detected using Super Signal® West Pico chemiluminescent substrate for HRP detection (Pierce) and FluorChem™ 8800 imaging system (Alpha Innotech).

2.5. Statistics

Results are expressed as the mean ± standard error of mean (S.E.M.). The significance of differences was calculated by analysis of variance supported by Dunnett's adjusted significance levels. A difference between treatment groups was considered significant when $P < 0.05$.

3. Results

3.1. LPS-induced TTP expression in J774 macrophages

Low levels of TTP mRNA were found in unstimulated J774 macrophages. When the cells were exposed to LPS (10 ng/ml) the amount of TTP mRNA was doubled in less than an hour, it peaked at 6 h, and declined thereafter (Fig. 1A). LPS (1–1000 ng/ml) induced TTP mRNA expression in a dose-dependent manner (Fig. 1B). Accordingly, increased TTP protein expression was found in LPS-treated cells (Fig. 1C). TTP protein levels were doubled in 3 h after addition of LPS, peaked at 6 h and declined thereafter, showing only slightly elevated levels at 24 h. In Western blot analysis of the TTP protein three immunoreactive bands between MWs of 36 and 46 kDa were found. This concurs with previous studies and is explained by different molecular sizes resulting from post-translational modifications of the molecule [18,19].

Pharmacological inhibitors were used to evaluate signaling pathways involved in LPS-induced TTP expression (Table 1). Dexamethasone (1 μM) had a clear effect and inhibited TTP mRNA expression by 76%. In addition, inhibitors of mitogen-activated protein kinase (MAPK) pathways reduced LPS-induced TTP mRNA expression. Extracellular signal-regulated kinase 1/2 (Erk1/2) inhibitor PD 98059 (10 μM) [20] inhibited TTP mRNA levels by 35% ($P < 0.05$). SP 600125 (10 μM), a novel anthrapyrazolone inhibitor of c-Jun N-terminal kinase (JNK) [21], reduced LPS-induced TTP mRNA by 43% ($P < 0.01$). An inhibitor of p38 MAPK, SB 203580 (1 μM) [22], inhibited TTP mRNA levels by 74% ($P < 0.01$) whereas SB 202474

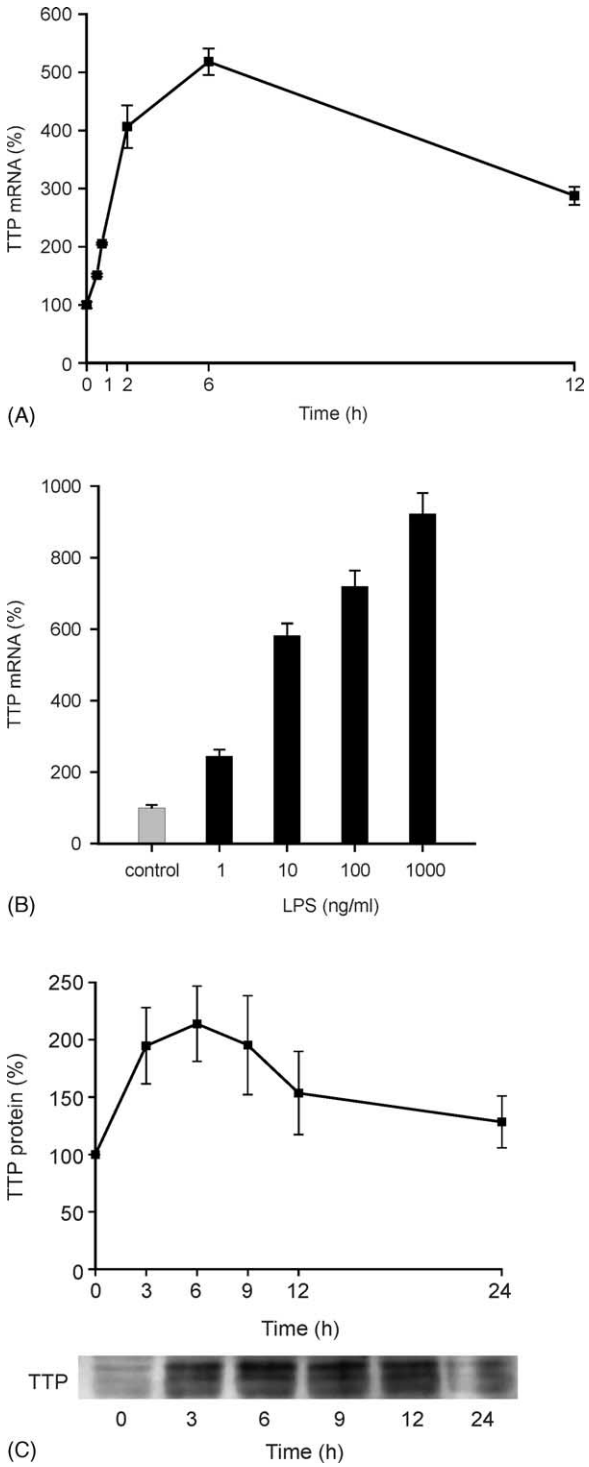

Fig. 1. The effect of LPS on TTP expression in J774 macrophages. (A) LPS (10 ng/ml) was used to stimulate macrophages and total RNA was extracted at the time points indicated. Quantitative PCR was used to measure TTP mRNA and the values were normalized to GAPDH mRNA. Values are mean ± S.E.M. ($n = 3$). (B) The cells were stimulated with the indicated amounts of LPS and total RNA was extracted after 6 h incubation. Quantitative PCR was used to measure TTP mRNA and the values were normalized to GAPDH mRNA. Values are mean ± S.E.M. ($n = 3$). (C) As in A, LPS was used to stimulate macrophages. Proteins were extracted at the time points indicated and TTP was measured by Western blot. Values are mean ± S.E.M. ($n = 5$).

Table 1
The effect of inhibitors of certain signaling pathways on LPS-induced TTP expression

Treatment	TTP mRNA (% of LPS-induced levels)	
Untreated	11 ± 1	
LPS (10 ng/ml)	100	
LPS + dexamethasone (1 μM)	24 ± 2	$P < 0.01$
LPS + PD 98059 (10 μM)	65 ± 7	$P = 0.047$
LPS + SP 600125 (10 μM)	57 ± 7	$P < 0.01$
LPS + SB 203580 (1 μM)	26 ± 3	$P < 0.01$
LPS + SB 202474 (1 μM)	96 ± 12	$P > 0.1$
LPS + PDTC (100 μM)	78 ± 9	$P > 0.1$
LPS + Cyclosporin A (10 μM)	87 ± 10	$P > 0.1$
LPS + Genistein (100 μM)	68 ± 8	$P = 0.082$
LPS + AG-490 (10 μM)	93 ± 13	$P > 0.1$

Cells were incubated for 6 h with or without LPS and the inhibitors of interest. Total RNA was extracted and subjected to quantitative PCR. Values are mean ± S.E.M. ($n = 3$), compared to samples treated with LPS only.

(a structural analog of SB 203580 that does not inhibit p38 [22]) had no effect on TTP mRNA expression.

Pyrrolidine derivative of dithiocarbamate (PDTC), cyclosporin A, genistein and tyrphostin AG-490 were used to evaluate the roles of nuclear factor κB (NF-κB), calcium/calmodulin-dependent phosphatase calcineurin, tyrosine phosphorylation and JAK-2 in TTP mRNA expression respectively. Genistein reduced LPS-induced TTP mRNA expression by 32 ± 8%, ($P = 0.082$) whereas none of the other compounds had any clear effect on TTP mRNA accumulation in LPS-treated J774 macrophages.

3.2. Dexamethasone and dissociated steroid RU24858 inhibited LPS-induced TTP expression in J774 macrophages

In subsequent studies, the inhibitory effect of dexamethasone on LPS-induced TTP expression was investigated in more detail. The inhibitory effect of dexamethasone on TTP mRNA accumulation was dose-dependent, and maximal inhibition was reached at drug concentrations of 100–10 000 nM (Fig. 2A). A similar dose-dependent inhibitory effect was also seen in TTP protein expression (Fig. 2B).

Dissociated glucocorticoids such as RU24858 are synthetic glucocorticoid ligands possessing different transactivation and transrepression profiles than those of classical anti-inflammatory steroids. RU24858 has been shown to have transrepression properties similar to those of dexamethasone on AP-1- and NF-κB-dependent gene expression, but it does not induce gene expression through glucocorticoid response element [23,24]. We tested the effect of RU24858 on TTP expression to find out if the TTP-reducing effect of dexamethasone was associated with the transrepression or transactivation mechanisms of glucocorticoids. RU24858 inhibited TTP mRNA expression in a dose-dependent manner, and maximal effect was reached at 100–1000 nM concentrations (Fig. 2C). Accordingly, an inhibitory effect on TTP protein expression was also found (Fig. 2D). These results suggest that the inhibitory action of glucocorticoids on TTP expression is mediated through their transrepression mechanisms.

In the presence of a glucocorticoid receptor (GR) antagonist, mifepristone (5 μM), dexamethasone or RU24858 did not reduce TTP mRNA expression in LPS-treated macrophages (Fig. 3). These data suggest that the effects of dexamethasone and RU24858 are mediated through the glucocorticoid receptor.

3.3. Dexamethasone and dissociated steroid RU24858 did not alter TTP mRNA half-life in LPS-treated J774 macrophages

Dexamethasone reduced LPS-induced TTP mRNA levels when measured at 3 h, 6 h or 9 h after the addition of LPS ± dexamethasone by 31%, 55% and 38% respectively (Fig. 4A). In the subsequent studies, the effects of dexamethasone and RU24858 on the half-life of TTP mRNA were investigated by actinomycin D assay. Cells were stimulated with LPS (10 ng/ml) in the presence or absence of dexamethasone (100 nM) or RU24858 (100 nM) for 6 h, and thereafter actinomycin D (0.5 μg/ml) was added into the culture to inhibit transcription. Total mRNA was isolated at 1 h intervals after actinomycin D addition, and the TTP mRNA levels were measured.

The half-life on TTP mRNA was ∼1 h in LPS-treated cells, and neither dexamethasone nor RU24858 altered the TTP mRNA half-life (Fig. 4B).

3.4. Histone deacetylase inhibitors reduced the effects of dexamethasone and RU24858 on TTP expression in LPS-treated J774 macrophages

The suppressive effects of anti-inflammatory steroids on certain genes are mediated through histone deacetylation [25]. We therefore studied the effects of dexamethasone and RU24858 on LPS-induced TTP mRNA expression in the presence and absence of trichostatin A (TSA) and apicidin, which inhibit histone deacetylases leading to histone hyperacetylation, and by that mechanism can abolish the effects of glucocorticoids on histone deacetylation.

In the absence of histone deacetylase inhibitors, dexamethasone (100 nM) and RU24858 (100 nM) inhibited LPS-induced TTP mRNA expression by 53% ($P < 0.05$) and 52% ($P < 0.05$) respectively (Fig. 5A). In the presence of TSA or apicidin the glucocorticoids did not significantly reduce TTP expression (Fig. 5B and C respectively). These results suggest that histone deacetylation and transcriptional silencing may mediate the inhibitory effects of dexamethasone and dissociated steroid RU24858 on TTP expression.

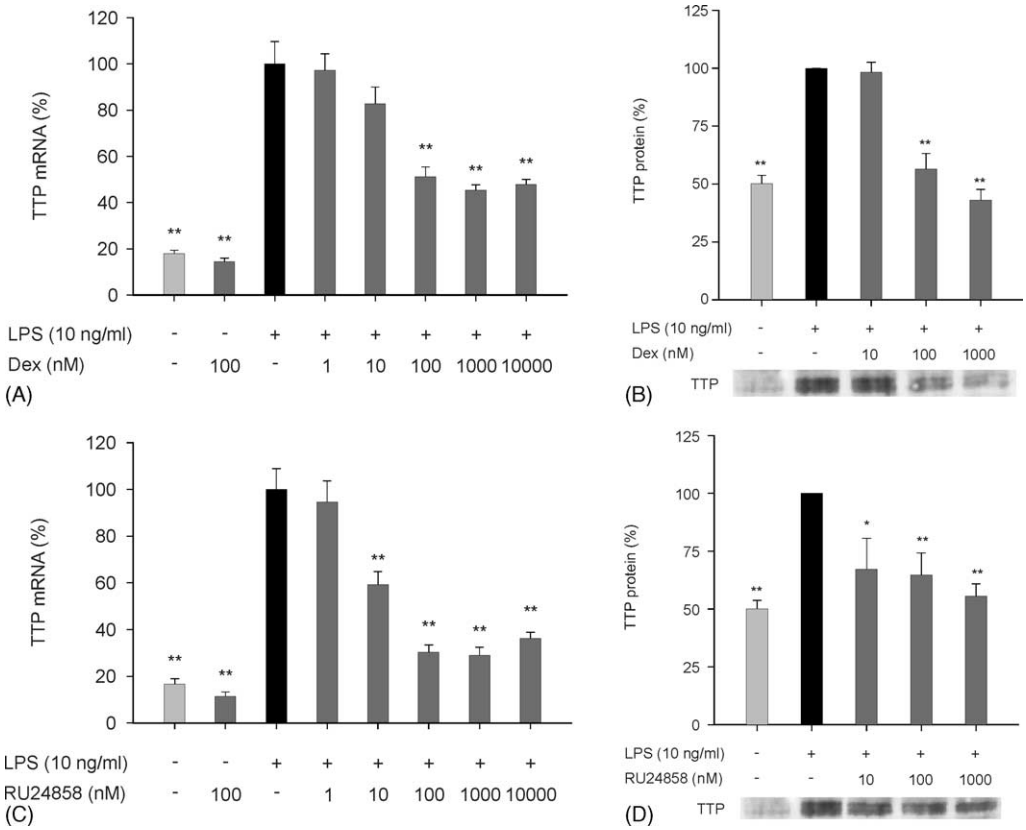

Fig. 2. The effect of dexamethasone (Dex) and RU24858 on LPS-induced TTP expression. Cells were incubated for 6 h with or without LPS and glucocorticoids. (A, C) Quantitative PCR was used to measure TTP mRNA and the values were normalized to GAPDH mRNA. Values are mean ± S.E.M. ($n = 3$–6). (B, D) TTP protein was analysed by Western blot. The gels are representatives of four separate experiments with similar results. Density values are mean ± S.E.M. ($n = 4$). $^{**}P < 0.01$, $^{*}P < 0.05$, when compared to samples treated with LPS only.

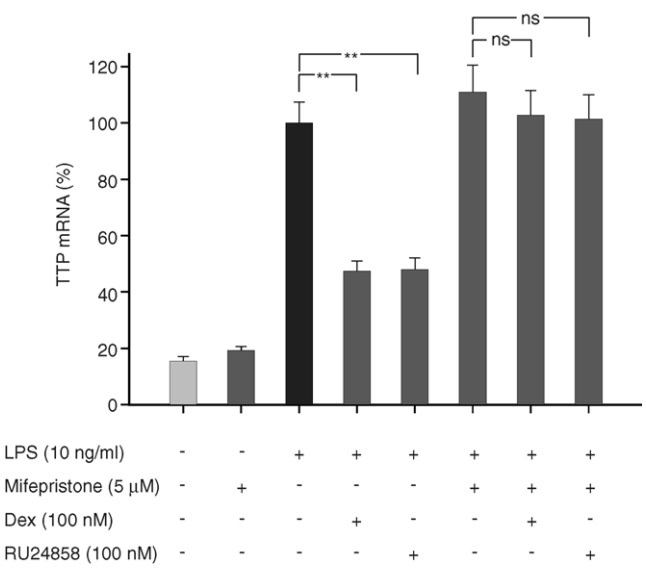

Fig. 3. The effect of glucocorticoid receptor antagonist mifepristone on TTP expression. Cells were cultured with LPS, mifepristone, dexamethasone (Dex) and RU24858 as indicated for 6 h and total RNA was extracted. Quantitative PCR was used to measure TTP mRNA and the values were normalized to GAPDH mRNA. Values are mean ± S.E.M. ($n = 4$). $^{**}P < 0.01$, ns = not significant, when compared to samples treated with LPS only or samples treated with the combination of LPS and mifepristone.

4. Discussion

In the present study, we found that dexamethasone and dissociated steroid RU24858 reduced LPS-induced TTP expression in a glucocorticoid receptor-mediated manner. The results suggest that the inhibitory effect of glucocorticoids on TTP expression was mediated through enhanced histone deacetylation and transcriptional silencing. In addition, the inhibitors of p38, JNK and Erk1/2 MAP kinases reduced the amount of TTP mRNA, suggesting a role for these kinases in the regulation of TTP gene expression.

In an inflammatory reaction, TTP is known to regulate the production of pro-inflammatory proteins TNF-α, granulocyte-macrophage colony-stimulating factor, interleukin-3 and cyclooxygenase-2 by destabilizing their mRNAs through A + U-rich elements [13–16]. In the present study we found that TTP is expressed in macrophages in response to bacterial LPS. This result is in line with the earlier findings that certain inflammatory stimuli and growth factors increase TTP expression [1,26], and suggests that TTP is a part of the endogenous machinery regulating the restriction and resolution of the inflammatory process.

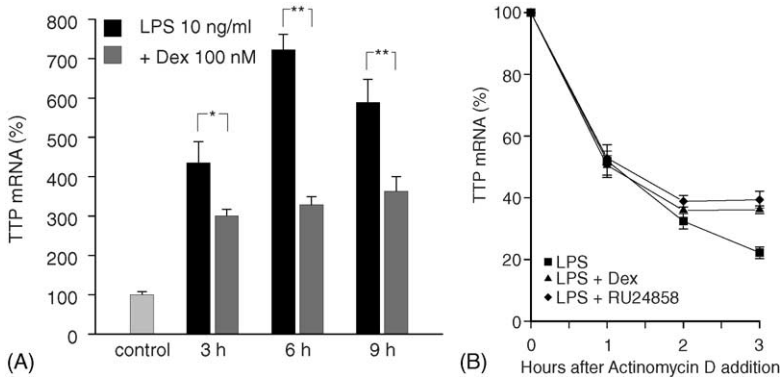

Fig. 4. The kinetics of the effect of dexamethasone on TTP mRNA expression. (A) J774 macrophages were treated with LPS (10 ng/ml) in the presence or absence of dexamethasone (Dex) (100 nM). Total RNA was extracted at the time points indicated. Quantitative PCR was used to measure TTP mRNA and the values were normalized to GAPDH mRNA. Values are mean ± S.E.M. ($n = 3$). $^{**}P < 0.01$, $^{*}P < 0.05$, when compared to samples treated with LPS only. (B) Macrophages were treated with LPS (10 ng/ml) with or without dexamethasone (100 nM) or RU24858 (100 nM) for 6 h, and then actinomycin D (0.5 μg/ml) was added to the cells. Cells were then harvested at indicated time points and total RNA was extracted and subjected to quantitative PCR. The mRNA level of all treatments at actinomycin D addition was set at 100%. Values are mean ± S.E.M. ($n = 3$).

The most interesting finding in the present study was that dexamethasone and dissociated steroid RU24858 inhibit TTP expression in a dose-dependent manner. To our knowledge this effect has not been reported earlier. A core glucocorticoid-like response element has been suggested to reside in TTP promoter [1]. The core glucocorticoid-like response element of TTP resembles more a glucocorticoid response element (GRE) than a negative GRE [27]. Based on these data and on our present results on the effects of dissociated steroid RU24858 it is not likely that the binding of glucocorticoid–GR complex to the core glucocorticoid-like response element is responsible for the inhibition of TTP expression, but rather that the inhibition is mediated through GRE-independent transrepression mechanisms.

Glucocorticoid receptor antagonist mifepristone competes with dexamethasone for the same binding site in glucocorticoid receptor and inhibits the effects of glucocorticoids [28,29]. In the present study, dexamethasone and RU24858 had no effect on TTP mRNA expression in the presence of mifepristone. These data suggest that the inhibitory effects of dexamethasone and RU24858 on LPS-induced TTP expression are mediated through GR.

Dexamethasone reduced TTP mRNA expression at early time points after the stimulus, and neither dexamethasone nor RU24858 altered the half-life of TTP mRNA. These data suggest that the effect of glucocorticoids occurs at the transcriptional level. Transcriptional activation is associated with histone acetylation, which facilitates gene transcription [30]. The inhibitory effects of glucocorticoids on the expression of certain genes are mediated through histone deacetylation [25]. In order to study if this is also the case in glucocorticoid-induced suppression of TTP expression, histone deacetylase inhibitors TSA and apicidin were added into the culture. TSA and apicidin reduced

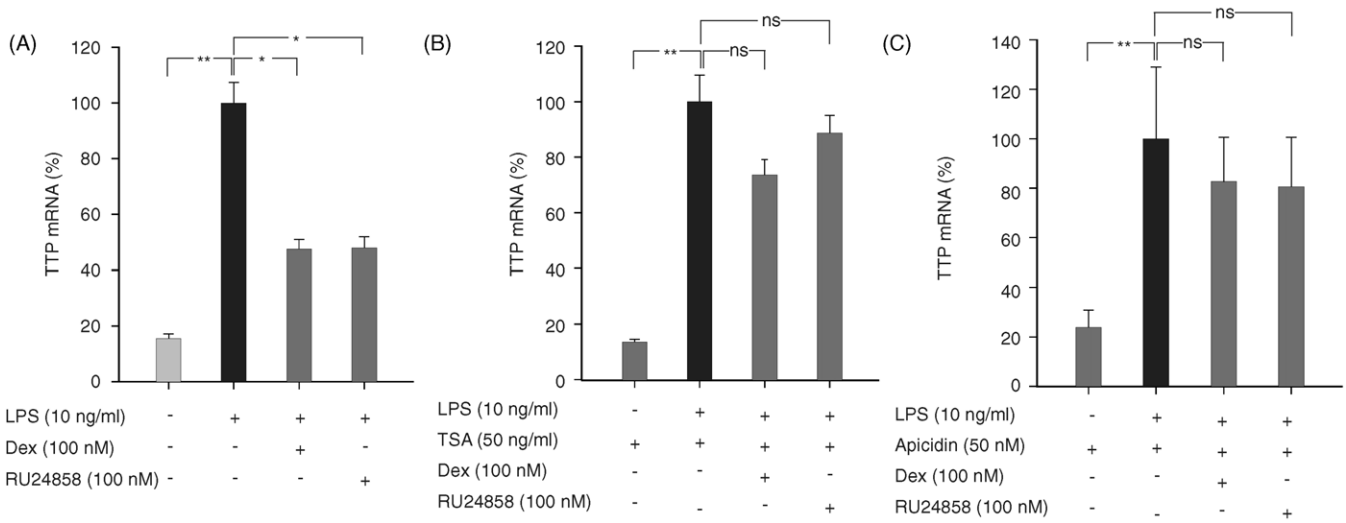

Fig. 5. The effect of histone deacetylase inhibitors TSA and apicidin on the suppressive effect of dexamethasone and RU24858 on LPS-induced TTP expression. Cells were incubated for 6 h with the compounds tested. Quantitative PCR was used to measure TTP mRNA and the values were normalized to GAPDH mRNA. Values are mean ± S.E.M. ($n = 4$). $^{**}P < 0.01$, $^{*}P < 0.05$, ns = not significant, when compared to samples treated with LPS or to samples treated with a combination of LPS and TSA or LPS and apicidin.

the effects of dexamethasone and RU24858 on TTP expression. These results suggest that the effects of dexamethasone and RU24858 on TTP expression are mediated through enhanced histone deacetylation. The mechanisms by which glucocorticoids induce histone deacetylation are not known in detail. It has been reported that GR is a direct inhibitor of CREB-binding protein (CBP)-associated histone acetyltransferase (HAT) activity and it also recruits histone deacetylase 2 to the CBP–HAT complex, and these two mechanisms may enhance histone deacetylation and transcriptional silencing [31]. However, the role of histone deacetylation as a mechanism of anti-inflammatory steroids to regulate the expression of TTP and other genes needs further mechanistic studies.

We also studied the involvement of some other signaling mechanisms in LPS-induced TTP expression by pharmacological means. PDTC (NF-κB inhibitor), cyclosporin A (calcineurin inhibitor) and AG-490 (JAK-2 inhibitor) had no effect, suggesting a minor effect of those signaling pathways in TTP expression. Although an NF-κB-like binding site has been described in TTP intron [32] it is not likely that NF-κB is a critical transcription factor for TTP as PDTC had no effect on TTP expression. By contrast, the signaling pathways regulating LPS-induced TTP mRNA expression seem to involve p38, JNK and Erk1/2 kinases. A specific inhibitor of p38, SB 203580 [22] inhibited the expression of TTP mRNA by 74%, whereas SB 202474, a structural analog of SB 203580, that does not inhibit p38 [22], had no effect on TTP expression. These results suggest that p38 positively regulates the expression of TTP. PD 98059 (an inhibitor of the Erk1/2 pathway) reduced the accumulation of TTP mRNA by 35% suggesting that Erk1/2 MAPK also has a role in the regulation of TTP expression. Our results on the involvement of Erk1/2 and p38 in the induction of TTP support the previous findings of Inuzuka et al. [33] and Mahtani et al. [18].

JNK inhibitor SP 600125 [21] reduced TTP mRNA levels by 43% in cells stimulated with LPS. This concurs with the recent findings of Brooks et al. [34] on the inhibitory effect of SP 600125 on TTP expression in THP-1 cells. In addition, cycloheximide (which among its other effects is a strong activator of JNK [35]) has been reported to cause superinduction of TTP [36–39]. These reports together support the role of JNK in the up-regulation of TTP expression.

The mechanisms by which MAP kinases regulate TTP expression are not known in detail. Mitogen- and stress-activated protein kinases-1 and -2 (MSK1, MSK2) have been shown to mediate the effects of p38 and Erk1/2 on the expression of many immediate early genes in inflammation [40] but their role in TTP expression remains to be studied.

Two recent articles evaluated the roles of MAPKs in TTP mRNA stability. Brooks et al. found that in THP-1 cells PD 98059 destabilized TTP mRNA, while SB202190 (an inhibitor of p38) and SP 600125 did not affect LPS-mediated TTP mRNA stability [34]. In contrast, Tchen et al. reported that another p38 inhibitor SB 203580 destabilized TTP mRNA in RAW264.7 cells [41]. Both reports also describe the involvement of TTP itself in the regulation of TTP mRNA stability [34,41]. In addition to the regulation of TTP expression, p38, JNK and Erk1/2 may affect the activity of TTP, as they have been shown to phosphorylate recombinant TTP in vitro [42].

In the present study, we show that LPS-induced TTP expression in macrophages is down-regulated by anti-inflammatory steroids and inhibitors of p38, JNK and Erk1/2 MAP-kinases. To our knowledge, this is the first report showing that dexamethasone and dissociated steroid RU24858 inhibit TTP expression. Our results suggest that the inhibition of TTP expression by glucocorticoids is mediated through histone deacetylation and transcriptional silencing in a glucocorticoid receptor-dependent manner.

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