Acute Phase Mediators Modulate Thrombin-activable Fibrinolysis Inhibitor (TAFI) Gene Expression in HepG2 Cells*

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Michael B. Boffa, Jeffrey D. Hamill, Deborah Maret, Darryl Brown, Michelle L. Scott, Michael E. Nesheim, and Marlys L. Koschinsky

From the Departments of Biochemistry and Medicine, Queen’s University, Kingston, Ontario K7L 3N6, Canada

Thrombin-activable fibrinolysis inhibitor (TAFI) has recently been identified as a positive acute phase protein in mice, an observation that may have important implications for the interaction of the coagulation, fibrinolytic, and inflammatory systems. Activated TAFI (TAFIa) inhibits fibrinolysis by removing the carboxy-terminal lysines from partially degraded fibrin that are important for maximally efficient plasminogen activation. In addition, TAFIa has been shown to be capable of removing the carboxy-terminal arginine residues from the anaphylatoxins and bradykinin, thus implying a role for the TAFI pathway in the vascular responses to inflammation. In the current study, we investigated the ability of acute phase mediators to modulate human TAFI gene expression in cultured human hepatoma (HepG2) cells. Surprisingly, we found that treatment of HepG2 cells with a combination of interleukin (IL)-1β and IL-6 suppressed endogenous TAFI mRNA abundance in HepG2 cells (−60% decrease), while treatment with IL-1α or IL-6 alone had no effect. Treatment with IL-1β and/or IL-6 had no effect on TAFI promoter activity as measured using a luciferase reporter plasmid containing the human TAFI 5′-flanking region, whereas treatment with IL-1β and IL-6 in combination, but not alone, decreased the stability of the endogenous TAFI mRNA. Treatment with the synthetic glucocorticoid dexamethasone resulted in a 2-fold increase of both TAFI mRNA levels and promoter activity. We identified a functional glucocorticoid response element (GRE) in the human TAFI promoter between nucleotides −92 and −78. The GRE was capable of binding the glucocorticoid receptor, as assessed by gel mobility shift assays, and mutation of this element markedly decreased the ability of the TAFI promoter to be activated by dexamethasone.

Thrombin-activable fibrinolysis inhibitor (TAFI) was first identified in 1989 by two independent groups as a basic carboxypeptidase present in fresh serum that was distinct from the constitutive basic carboxypeptidase N (1, 2). By virtue of the intrinsic instability of this enzyme, whose activity disappeared within 2 h upon incubation at 37 °C, Hendriks et al. (1) designated the novel activity "unstable" carboxypeptidase or carboxypeptidase U (1). Campbell and Okada (2) determined that the enzyme removed arginine residues from substrates more efficiently than lysines and therefore designated it carboxypeptidase R (2). In 1991, Eaton et al. (3) isolated a cDNA encoding the zymogen form of the enzyme and found that it was highly homologous to pancreatic carboxypeptidase B. Bajzar et al. (4) independently isolated a protein on the basis of its ability to inhibit fibrinolysis in the setting of sustained activation of the coagulation cascade; on the basis of this property, they named the protein TAFIa. Amino acid sequence analysis of TAFI revealed it to be identical to plasma carboxypeptidase B and carboxypeptidases U and R. TAFI can be activated by thrombin (4), plasmin (5), and thrombin in complex with thrombomodulin (6), with the last being by far the most efficient activator. Activated TAFI (TAFIa) inhibits fibrin clot lysis by removing the carboxy-terminal lysine residues from partially degraded fibrin that mediate positive feedback in the fibrinolytic cascade (7). As such, it has been hypothesized that TAFI plays a role in vivo in mediating the balance between coagulation and fibrinolysis.

Additional substrates for TAFIa have been identified that imply a role for the TAFI pathway beyond inhibition of fibrinolysis. TAFIa has been shown to remove the carboxy-terminal arginines from the anaphylatoxins C3a and C5a (8) as well as from bradykinin (9–11). As such, TAFIa may modulate inflammatory processes in the vasculature in the setting of activation of the coagulation cascade. Additional evidence for a role for the TAFI pathway in inflammation comes from the recent observation that TAFI is an acute phase protein in mice; injection of the animals with bacterial lipopolysaccharide (LPS) elicited increases in both plasma TAFI concentrations and hepatic TAFI mRNA abundance (12). In order to begin to assess if human TAFI is also an acute phase protein and to determine the molecular mechanisms underlying this potential phenomenon, we have studied the ability of acute phase mediators to alter TAFI gene expression in a cultured human hepatoma cell model.

EXPERIMENTAL PROCEDURES

Materials—Restriction and modification enzymes were from New England Biolabs, Invitrogen, Promega, and Stratagene. [γ-32P]ATP, [α-32P]dATP, and poly(dIdC)-(dIdC) was purchased from Amersham Biosciences. TRizol reagent, minimal essential medium (MEM), Dulbecco’s modified Eagle’s medium/nutrient mixture F-12 (DMEM/F-12), and penicillin-streptomycin-Fungizone (PSF) were obtained from Invitrogen. Fetal calf serum was purchased from ICN. NucleoTrap mRNA purification kits were from Clontech. Synthetic oligonucleotides were purchased from Cortec DNA Service Laboratories, Inc. (Kingston, Ontario, Canada). IL-1β, IL-6, dexamethasone, and actinomycin C1 were defrayed in part by the payment of page charges. This article must be hereby marked “advertisement.”

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were purchased from Sigma and were reconstituted as recommended by the manufacturer. Recombinant human glucocorticoid receptor expressed in insect cells and a double-stranded oligonucleotide corresponding to the human tyrosine aminotransferase glucocorticoid response element (TAT-GRE) was obtained from Affinity Bioreagents, Inc. A cDNA clone corresponding to the full-length mRNA encoding the human γ-chain of fibrinogen was obtained from the American Type Culture Collection.

**Cell Culture and RNA Analysis**—HepG2 cells (human hepatocellular carcinoma) (13) were grown in MEM containing 10% fetal calf serum and 1% PSF. Cells were maintained in a humidified 37°C incubator under a 95% air, 5% CO2 atmosphere. Cytokine(s)/and/also/dexamethasone were added to the culture medium at various times during the 42-h incubation period. The cells were washed three times in phosphate-buffered saline and given fresh medium, and incubation was continued for an additional 42 h. In some experiments, hormones or cytokines were added to the culture medium at various times during the 42-h incubation period. The cells were harvested for preparation of cytoplasmic extracts for luciferase and β-galactosidase assays as previously described (18). For each sample, the relative luciferase activity was calculated to be the luciferase activity per unit of β-galactosidase activity per unit volume of cell extract.

**Acute Phase Mediators and TAFI Expression**

For luciferase reporter gene assays, HepG2 cells were grown in 6-well plates (Corning) and transfected by the method of calcium phosphate co-precipitation (22). Typically, 100,000 cells (in 0.2 ml of partially purified recombinant human GR) was constructed as follows: pEGFP-GRwtN525 (pEGFP-C1 (Clontech) containing a rat GR fragment encompassing amino acids 22-525) was digested with MluI downstream of the simian virus 40 (SV40) polyadenylation site), the ends were made blunt with T4 DNA polymerase, and the plasmid was then digested with BglII (within the rat GR coding sequence). The resultant fragment was inserted into pGR-PTL2 in which the corresponding sequences of the wild-type receptor cDNA were removed by digestion with BamHI (at which point the ends were made blunt) and BglII. Expression of all rat GR variants in mammalian cells was driven by the SV40 early promoter with the exception of the rat GR-L501P, whose expression was driven by the Rous sarcoma virus (RSV) long terminal repeat.

**Reporter Gene Assays**—For luciferase reporter gene assays, HepG2 cells were grown in 6-well plates (Corning) and transfected by the method of calcium phosphate co-precipitation (22). Typically, 100,000 cells (in 0.2 ml of partially purified recombinant human GR) was constructed as follows: pEGFP-GRwtN525 (pEGFP-C1 (Clontech) containing a rat GR fragment encompassing amino acids 22-525) was digested with MluI downstream of the simian virus 40 (SV40) polyadenylation site), the ends were made blunt with T4 DNA polymerase, and the plasmid was then digested with BglII (within the rat GR coding sequence). The resultant fragment was inserted into pGR-PTL2 in which the corresponding sequences of the wild-type receptor cDNA were removed by digestion with BamHI (at which point the ends were made blunt) and BglII. Expression of all rat GR variants in mammalian cells was driven by the SV40 early promoter with the exception of the rat GR-L501P, whose expression was driven by the Rous sarcoma virus (RSV) long terminal repeat.

For each sample, the relative luciferase activity was calculated to be the luciferase activity per unit of β-galactosidase activity per unit volume of cell extract.

**Results**—Complementary sets of oligonucleotides encompassing the putative GRE in the TAFI promoter were synthesized: sense 5'-CAC AGG AAC AAG AGG GAA CAT GCC GTC ATA TTT TTA CC-3'; antisense 5'-GTT TAA AAT ATA ACA GCA TGT TCC GGA CTG TCC CTC CTG-3' (within the rat GR coding sequence). The resultant fragment was inserted into pGR-PTL2 in which the corresponding sequences of the wild-type receptor cDNA were removed by digestion with BamHI (at which point the ends were made blunt) and BglII. Expression of all rat GR variants in mammalian cells was driven by the SV40 early promoter with the exception of the rat GR-L501P, whose expression was driven by the Rous sarcoma virus (RSV) long terminal repeat.

**Effect of Acute Phase Mediators on TAFI Gene Expression**—It has been demonstrated that injection of mice with bacterial lipopolysaccharide (LPS) results in an increase in both plasma TAFI concentrations as well as hepatic TAFI mRNA abundance (12). To determine which mediators of the acute phase response regulate TAFI gene expression, we treated human hepatoma (HepG2) cells for 24 h with the cytokines interleukin (IL)-1β and/or -6, as well as dexamethasone (a synthetic glucocorticoid hormone analog). We measured the abundance of the endogenous TAFI mRNA by Northern blot analysis, using the acid ribosomal phosphoprotein PO (36B4) mRNA as an internal standard (Fig. 1). While we found that TAFI mRNA
abundance was actually decreased by treatment of the cells with a combination of IL-1β and IL-6, IL-1β alone or IL-6 either alone or in combination with dexamethasone had little or no effect. Treatment with dexamethasone resulted in dose-dependent changes in TAFI mRNA abundance: an increase of up to 2-fold was observed that peaked at doses between 0.5 and 2 μM hormone.

To verify that our cell culture model was a valid model for the analysis of the effect of acute phase mediators on TAFI gene expression, we measured the abundance of the mRNA for the γ-chain of fibrinogen (γ-Fgn) under similar conditions. As has been previously reported (24), both IL-6 and dexamethasone induce expression of γ-Fgn, with the greatest effect occurring when the two mediators are administered in combination. The magnitude of the induction by IL-6 plus dexamethasone was, however, less than has been reported for the mRNA levels of IL-1β and IL-6 in combination to decrease TAFI mRNA abundance did not seem to result from a decrease in TAFI promoter activity, we investigated whether these cytokines could influence the stability of the TAFI mRNA transcript. HepG2 cells were treated for 24 h with IL-1β and/or IL-6 at which time transcription was arrested by the addition of actinomycin C. RNA was harvested at different times after the addition of actinomycin C, and the remaining TAFI mRNA was quantified by Northern blot analysis (Fig. 3). The results show that the TAFI mRNA has an intrinsic half-life of about 3 h, identifying the TAFI transcript as a relatively short-lived mRNA species (28); addition of IL-1β and IL-6 in combination results in a destabilization of the TAFI mRNA whereas either cytokine administered alone had no effect. From regression of the Northern blot data, the effect of the combined cytokines results in a 22% decrease in the half-life of the TAFI transcript.

Identification of a Glucocorticoid Response Element in the TAFI Promoter—In order to identify sequences in the TAFI promoter that mediate the increase in promoter activity elicited by dexamethasone, we utilized a series of luciferase reporter plasmids containing progressive 5′-deletions of 5′-flanking sequence (Fig. 4A). The plasmids were transiently transfected into HepG2 cells, and the cells were incubated either in the absence or presence of 1.0 μM dexamethasone for 42 h prior to harvest and luciferase assay. Full responsiveness to dexamethasone was preserved upon deletion of 5′-flanking sequence up to nucleotide −100 (the numbering refers to the number of nucleotides upstream of one of the transcription start sites in the TAFI promoter) (Fig. 4B). However, deletion
Actinomycin C was added to 5 HEPG2 cells were treated with IL-1 before the addition of actinomycin C, and the abundance of remaining TAFI mRNA quantitated by Northern blot analysis. The data shown are the mean S.E. of three independent experiments.

Inspection of the sequence downstream of promoters of target genes thereby stimulating their transcription. Hormone-bound GR binds as a homodimer to specific sequences, known as GREs, in the promoter, we performed co-transfection experiments with the GR for dexamethasone-dependent activation of the TAFI promoter revealed the presence of a sequence, between -92 and -78, that resembles a consensus GRE (30) in that it is an imperfect inverted repeat with a 3-nucleotide spacing of the 6-nucleotide half-sites (Fig. 5A). Mutations were introduced into this candidate GRE that would be expected to abolish its ability to bind GR (Fig. 5A). HepG2 cells were transfected with a luciferase reporter plasmid encompassing the mutations and including the TAFI 5'-flanking sequence up to -1128 (TAFI[−1128ΔGRE]-luc), or the corresponding wild-type reporter plasmid (TAFI[−1128]-luc), and the cells were treated with dexamethasone (Fig. 5B). The mutations greatly decreased the response of the TAFI promoter; interestingly, the decrease in the extent of the response was similar to that observed upon deletion of sequences between -100 and -90.

Glucocorticoid hormones exert their effects on transcription through binding to and activating the GR, a member of the nuclear receptor superfamily (29). Ligand-bound GR binds as a homodimer to specific sequences, known as GREs, in the promoters of target genes thereby stimulating their transcription. Deletion of sequences up to -80 does not result in a further decrease in dexamethasone responsiveness, while deletion of sequences up to -73 results in a complete loss of TAFI promoter activity, as we have previously reported (18).

In order to examine the requirement for DNA binding of the GR for dexamethasone-dependent activation of the TAFI promoter, we performed co-transfection experiments with the wild-type or ΔGRE mutant TAFI promoter reporter plasmid and expression plasmids for rat GR (rGR) variants. The variants used (see Fig. 6A) included the full-length wild-type receptor (rGR-wt), a variant including only the amino-terminal 525 amino acids (i.e. lacking the DNA- and ligand-binding domains; rGR-N525), a variant including only the carboxyl-terminal 540 amino acids fused to the GAL4 DNA binding domain (i.e. lacking the amino-terminal transactivation domain and the DNA binding domain of the GR; rGR-540C), and a mutant of the full-length GR with a single point mutation that abolishes DNA binding (rGR-L501P).

While ectopic expression of the intact, full-length rGR substantially increased the magnitude of dexamethasone induction of the wild-type TAFI promoter (Fig. 6B), ectopic expression of the rGR variants lacking the ability to bind DNA had little or no influence on the magnitude of induction. Expression
The acute phase reaction is a complex host defense mechanism that, in response to triggers such as trauma, surgery, tissue infarction, or severe infection, aims to counteract the responding to the wild-type TAFI GRE and recombinant human GR expressed in insect cells (Fig. 7A). As a positive control, gel mobility shift assays were also performed using the GRE from the TAT gene (Fig. 7B). The autoradiograms show a complex of low mobility is formed using the probe containing the wild-type TAFI-GRE as well as the TAT-GRE. These complexes represent specific binding of the GR because they are competed effectively by an excess of unlabeled oligonucleotides containing the TAT-GRE and the wild-type TAFI-GRE but not effectively by the mutant TAFI-GRE (TAFI-ΔGRE). No specific complexes were observed when radiolabeled oligonucleotides containing the mutant TAFI GRE were utilized (data not shown).

**DISCUSSION**

The acute phase reaction is a complex host defense mechanism that, in response to triggers such as trauma, surgery, tissue infarction, or severe infection, aims to counteract the
underlying challenge while restoring homeostasis (reviewed in Refs. 31 and 32). Among the features of the acute phase response are systemic changes such as fever, increases in neutrophil production, changes in lipid and amino acid metabolism and activation of the coagulation and complement cascades as well as changes in the expression of a panel of liver-expressed plasma proteins. These acute-phase proteins are classified as either positive or negative acute phase proteins depending on whether their expression is induced or repressed, respectively, in the acute phase. Among the acute phase proteins are C-reactive protein, serum amyloid A, α1-acid glycoprotein, components of the complement cascade, proteins of the coagulation and fibrinolytic cascades, protease inhibitors, and proteins involved in transport and inflammatory functions. The magnitude of the changes in expression can be small (such as a 50% increase in the expression of ceruloplasmin) to vast (such as a greater than 1000-fold increase in the expression of C-reactive protein and serum amyloid A).

Regulation of the expression of acute phase proteins is most often at the level of their transcription in liver and is largely a function of the action of certain inflammatory cytokines (reviewed in Ref. 33). Acute phase proteins are divided into two broad categories based on the cytokines that regulate their expression: class I proteins, including serum amyloid A, C-reactive protein, complement factor C3, and α1-acid glycoprotein, are induced by IL-1-type cytokines in concert with IL-6-type cytokines; class II proteins, including fibrinogen, haptoglobin, and α2-macroglobulin, are induced by IL-6-type cytokines with the participation of glucocorticoid hormones.

A study in which mice were injected intraperitoneally with a lethal dose of bacterial lipopolysaccharide, a maneuver that would be expected to provoke a robust acute phase response, resulted in increases in concentrations of TAFI in plasma as well as in hepatic TAFI mRNA abundance (12). This study, therefore, identified TAFI as a positive acute phase protein. A role for the TAFI pathway in the acute phase response is reasonable to expect since this pathway may impact both on hemostatic as well as inflammatory functions. Activation of TAFI, with the attendant inhibition of fibrinolysis (4, 7), may stabilize clots formed in response to tissue damage or as a means to isolate regions of severe infection. On the other hand, the TAFI pathway may influence the vascular responses to inflammatory stress: the ability of TAFI(a) to remove the carboxyl-terminal arginine residues from C3a and C5a (the anaphylatoxins) and from bradykinin could have effects on vascular tone and permeability (8–11).

The potential increase in plasma TAFI concentrations in the acute phase may reflect a requirement for enhanced activity of the TAFI pathway during host defense. Alternatively, the activation of the coagulation and fibrinolytic systems that occurs during the acute phase may result in consumption of the existing pool of plasma TAFI that could be compensated for by an increase in hepatic TAFI expression. Further analysis of the TAFI pathway during the acute phase will likely yield valuable insights into the role of this pathway in regulating the balance between coagulation and fibrinolysis and in mediating interactions between the coagulation and inflammatory systems.

We have utilized a cultured human hepatoma cell model to assess if TAFI gene transcription is induced by acute phase mediators and to investigate the molecular mechanisms underlying these effects. HepG2 cells represent a well characterized model system for the study of transcriptional regulation in the acute phase, as they retain many of the characteristics of hepatocytes, endogenously express many liver-specific genes, and contain cell surface receptors for the relevant inflammatory cytokines (13, 33). Surprisingly, we found that treatment
of HepG2 cells with IL-1β and IL-6 in combination reduced expression of the endogenous TAFI gene, as assessed by Northern blot analysis (Fig. 1). However, we did not observe an effect of this combination of cytokines on TAFI promoter activity measured by transient transfection of luciferase reporter plasmids (Fig. 2). Indeed, we found that these two cytokines, when administered in combination but not alone, were capable of decreasing the stability of the TAFI mRNA, which likely accounts for the ability of these cytokines to decrease TAFI mRNA abundance. We hypothesize that IL-1β and IL-6 together modulate the expression of a factor or factors that influences TAFI mRNA decay. IL-6 or IL-1β alone had no effect on TAFI mRNA abundance in HepG2 cells or TAFI promoter activity (Figs. 1 and 2). However, dexamethasone treatment, either in the presence or absence of IL-6, increased TAFI promoter activity ~2-fold; dexamethasone alone increased TAFI mRNA abundance almost 2-fold but had no effect in the presence of IL-6.

We identified a functional glucocorticoid response element in the TAFI promoter between −92 and −78; mutation of this GRE greatly decreased the ability of the TAFI promoter to be activated by dexamethasone (Fig. 5B) and of the TAFI-GRE to bind to the GR (Fig. 7). Of note, the unlabeled TAFI-GRE was a less effective competitor for binding of GR to either probe than unlabeled positive control TAT-GRE (Fig. 7), suggesting that the latter GRE possesses a higher affinity for GR. Indeed, the TAFI GRE contains numerous key substitutions in the downstream half-site sequence (Fig. 5A), relative to the consensus GRE (30) and the TAT-GRE, which differs from the consensus at only one position in the upstream half-site (TG-TACAGGATGTTC). In addition, a small but detectable extent of competition by the unlabeled TAFI-AGRE was observed with both the TAFI-GRE and TAT-GRE probes (Fig. 7), suggesting that the TAFI-AGRE retains a weak affinity for the GR. Indeed, the TAFI promoter containing the mutant GRE retains a small extent of dexamethasone inducibility (Fig. 5B) and exhibits dexamethasone-independent induction by ectopic expression of the constitutively active N525 GR variant, albeit to a reduced extent relative to the wild-type promoter (Fig. 6B). On the other hand, ectopically expressed full-length GR abolishes the small extent of dexamethasone inducibility of the mutant TAFI promoter (Fig. 6B). An explanation for this finding might be that the overexpressed full-length receptor consumed factors required for dexamethasone-dependent transactivation of the mutant TAFI promoter. That the TAFI-GRE is not an optimal GR binding sequence is perhaps consistent with the relatively modest extent to which dexamethasone induces transcription of the TAFI promoter, compared with the TAT promoter (e.g. 8–10-fold increase in TAT mRNA in primary rat hepatocytes induced by 1 μM dexamethasone, Ref. 34).

In the study in mice that identified TAFI as a positive acute phase protein (12), the magnitude of the increase in hepatic TAFI mRNA abundance was not carefully measured; as such, it is difficult to directly compare it to our in vitro data. Nonetheless, it is perhaps surprising that the acute phase mediators we examined either increased TAFI gene expression by a relatively modest amount (~2-fold increase mediated by dexamethasone) or even decreased TAFI gene expression (~60% decrease mediated by IL-1β or IL-6). However, it is important to stress that even small changes in plasma concentrations of TAFI can be expected to impact on the potential of the TAFI pathway to influence fibrinolysis. The range of plasma concentrations of TAFI (the upper limit of which has been reported to exceed 200–400 nM; Refs. 35–37) is well below the Kₘ for activation of TAFI by thrombin or thrombin-thrombomodulin (~1 μM; Ref. 6); as such, a change in concentration of TAFI in plasma would result in a corresponding change in the rate of TAFI activation by thrombin or thrombin-thrombomodulin. Indeed, a strong correlation between plasma TAFI concentrations and in vitro clot lysis times has been observed (35, 37).

No data currently exist that explicitly address potential changes in TAFI plasma concentrations in the acute phase in humans, although associations between TAFI concentrations and those of fibrinogen (38, 39) and C-reactive protein (38) have been reported, suggesting that TAFI gene expression could be positively regulated by inflammatory mediators. Intracellular signals elicited by IL-1 ultimately result in the activation of certain transcription factors, specifically AP-1, NF-κB, and C/EBPβ (33). IL-6 signaling results in the activation of the transcription factor STAT3 (33). Functional cooperation between GR and all of these transcription factors has been reported (40–44). In addition to the functional GRE, we have identified a functional C/EBP binding site between −52 and −40 of the TAFI promoter (45). Examination of the TAFI gene 5′-flanking sequence for consensus transcription factor binding sites using MatInspector (46) revealed potential binding sites for AP-1 but not STAT3 or NF-κB (data not shown). Alternatively, it cannot be ruled out that in humans, TAFI is either not an acute phase protein or is a negative acute phase protein. Different acute phase proteins have distinct temporal patterns of induction and subsequent return to baseline (31); the temporal pattern of TAFI expression during the acute phase could reflect the changing balance between cytokine and glucocorticoid signaling pathways over time.

The association of TAFI concentrations with inflammatory markers and fibrinogen suggests a role for glucocorticoid hormones (or other inflammatory mediators) in regulating plasma TAFI levels in the setting of chronic inflammation. For example, one study found that compared with preoperative plasma TAFI levels in patients requiring coronary artery bypass grafting (which were higher than in healthy controls), postoperative plasma TAFI levels fell 17% by day 3, then rose again 14% by day 6 (38). On the other hand, glucocorticoid hormones are important clinically as anti-inflammatory drugs. Interestingly, one study compared the distribution of plasma TAFI concentrations in a sample of patients with rheumatoid arthritis with that in a healthy control population: plasma TAFI concentrations are clearly higher in patients with rheumatoid arthritis, although the use of glucocorticoids in the patients was not accounted for (36).

In conclusion, we have documented the effect of acute phase mediators on endogenous TAFI gene expression in HepG2 cells by Northern blot analysis as well as on TAFI promoter activity by transient transfection into HepG2 cells of luciferase reporter plasmids harboring the TAFI 5′-flanking region. We found that when administered in combination, IL-1β and IL-6 decreased TAFI mRNA abundance by 60%, an effect that was associated with a destabilization of the TAFI mRNA transcript. Dexamethasone resulted in a 2-fold increase in both TAFI mRNA abundance and promoter activity, and we were able to identify a functional GRE in the TAFI promoter. Further studies will be required to fully elucidate the significance of these observations with respect to regulation of TAFI gene expression in the acute phase and other inflammatory states.

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