Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical Pharmacology 68 (2004) 1371–1381
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Determination of interleukin-4-responsive region in the human cytochrome P450 2E1 gene promoter

Ziad Abdel-Razzak[a], Michèle Garlatti[b], Martine Aggerbeck[b], Robert Barouki[b,*]

[a]*Faculty of Sciences I, Lebanese University, BP14/6573, Lebanon*
[b]*UMRS-Unit-490-INSERM, Université René Descartes, 45, rue des Saints-Pères, 75270 Paris Cedex 06, France*

Received 15 March 2004; accepted 4 June 2004

Abstract

Cytochrome P450 2E1 (CYP2E1) gene expression is known to be induced by interleukin-4 (IL4) and repressed by inflammatory cytokines, such as interleukin-1β3 (IL1β3) in human hepatocytes. The mechanisms involved in these transcriptional regulations remain elusive. In order to study these mechanisms, various constructs of the human CYP2E1 promoter were prepared and transfected into the human HepG2 hepatoma cell line. Our findings revealed that an IL4-responsive region of 128 bp (−671/−544) was required to mediate induction by IL4. IL1β caused moderate but significant decrease of the promoter activity, which was abolished when the two cytokines were combined. The IL1β inhibitory effect is mediated through a regulatory sequence independent of that of IL4. Furthermore, by using specific signaling pathway inhibitors, we demonstrated that IL4 activation required protein kinase C (PKC) activation. In addition, our results suggest that induction by IL4 was not dependent on a single binding site but rather on a complex region which includes putative binding sites for signal transducer and activator of transcription (STAT)6, activator protein (AP)-1, nuclear factor kappa-B (NFκB), nuclear factor of activated T cells (NFAT) and CCAAT enhancer binding protein (C/EBP). Electrophoretic mobility shift assays suggest that AP1 and NFAT transcription factors are able to bind to three sites in the IL4-responsive region.

Keywords: Cytochrome P450 2E1; CYP2E1; Interleukin-4; Interleukin-lβ; HepG2 cell line; Signal transduction; Transcription factors

1. Introduction

Cytochrome P450 2E1 (CYP2E1) is among the most important phase I xenobiotic metabolizing enzymes since it is involved in the metabolism of a wide spectrum of drugs, solvents and environmental procarcinogens. Moreover, it metabolizes endogenous substrates and plays a role in gluconeogenesis. CYP2E1 is mainly expressed in the liver but it is significantly expressed in other tissues. Apart from its transcriptional activation after birth, CYP2E1 expression is mainly controlled at the post-transcriptional level. For instance, stability of the CYP2E1 protein is regulated by ethanol, acetone and pyrazole. Patho-physiological states such as diabetes, prolonged starvation, obesity and insulin treatment regulate its mRNA stability [1]. In addition, triiodothyronine increases CYP2E1 mRNA stability of the rabbit CYP2E1 gene expressed in HepG2 cells [2]. Consequently, transcriptional regulation of CYP2E1 has been poorly studied and understood although its gene and cDNA have been cloned two decades ago [3,4].

Similarly to many xenobiotic metabolizing enzymes, CYP2E1 gene expression is inhibited during inflammation and infection. Such a decrease is mediated by cytokines. In fact, the pro-inflammatory cytokines IL1β, IL6 and tumor necrosis factorα (TNFα) down-regulate CYP2E1 gene expression. In contrast, interleukin-4 (IL4) up-regulates human CYP2E1 gene expression [5] as well as the mRNA level of glutathion-*S*-transferases-α [6]. CYP2E1 induction

Abbreviations: AP1, activator protein-1; C/EBP, CCAAT enhancer binding protein; CREB, cAMP response element-binding protein; CYP2E1, cytochrome P4502E1; DMEM, Dulbecco's modified eagle medium; H7, 1-[5-isoquinolinesulfonyl]-2-methylpiperazine; IL, interleukin; IRS, insulin receptor substrate; JaK, Janus kinase; LY294002, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one; MAP, mitogen activated protein; NFAT, nuclear factor of activated T cells; NFkB, nuclear factor kappa-B; PBS, phosphate buffer saline; PD98059, 2′-amino-3′-methoxyflavone; PI, phosphatidyl inositol; PKC, protein kinase C; Rapa, rapamycin; STAT, signal transducer and activator of transcription; TK, thymidine kinase; TNF, tumor necrosis factor

* Corresponding author. Tel.: +33-1-42-86-20-75; fax: +33-1-42-86-20-72.
E-mail address: Robert.Barouki@univ-paris5.fr (R. Barouki).

0006-2952/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2004.06.003

by IL4 occurs both at the mRNA and protein levels. A recent study demonstrated that the mRNA increase results, at least in part, from a 3.5-fold increase of its transcription rate [7]. IL4-mediated CYP2E1 induction seems to be specific to the human gene since IL4 was ineffective on the rat gene ([8], Abdel-Razzak et al., unpublished results) and on the rabbit one transfected into HepG2 cells [9]. In this later report, CYP2E1 promoter activity was induced by IL1α whereas IL1β was ineffective. The rat CYP2E1 promoter activity was decreased by IL1β and other inflammatory cytokines [8] but this effect seemed to be tissue-specific since IL1β has been reported to stimulate the expression of CYP2E1 in rat brain primary cortical glial cultures [10]. Species-specific regulation by cytokines is certainly due to divergence of the regulatory sequences since the rat CYP2E1 gene promoter shows 35–60% homology with the human one [11]. However, the diversity of signal transduction pathways mediating the effects of cytokines contributes also to their species- and tissue-specific effects. The molecular mechanisms as well as the *cis*-acting elements and *trans*-acting factors mediating CYP2E1 regulation by cytokines are still largely unknown and require intensive investigation.

IL4 is an immunomodulatory cytokine secreted by TH2 cells, basophils and mast cells. It is involved in diverse biological roles such as maturation and proliferation of immune cells and control of immunoglobulin class switching (review in [12]). IL4 is also known for its anti-tumoral properties [13] and can play anti-inflammatory roles [14,15]. IL4 acts via membrane receptors present on most cell types in vivo and in vitro. The type I and type II receptors consist of the IL4 receptor α-chain that heterodimerizes with either the IL2 receptor common γ-chain (γc) or the IL13 receptor αl chain [16,17]. Both heterodimers are devoid of intrinsic kinase activity, bind IL4 and transduce its signal. The signal transduction pathway upon ligand binding is not unique and differs according to the cell and receptor type [18,19]. The receptor, which is usually associated with a Janus kinase (JaK), undergoes tyrosine phosphorylation and, when activated, leads to increased tyrosine phosphorylation of cytosolic substrates such as signal transducer and activator of transcription (STAT)6 and insulin receptor substrate (IRS) −1 and 2. In addition to these signaling pathways, IL4 signaling may also occur via PI3 kinase and mitogen activated protein (MAP) kinase pathways [12]. Moreover, an increase of intracellular calcium and cAMP [20] as well as an increase of the oxidative stress [21] or protein kinase C (PKC) activation [22,23] can also mediate the IL4 action. These various signal transduction pathways account for the pleiotropic and differential effects exerted by IL4 on different cell types [24]. IL4-induced gene transcription is mediated by *cis*-acting regulatory regions, corresponding either to a simple STAT6 binding site [25] or to a complex DNA stretch where several regulatory proteins cooperate [26].

With respect to the induction of CYP2E1 gene expression by IL4, its mechanism of action and the transduction pathway remain elusive. The IL4 target DNA sequences that mediate CYP2E1 transcriptional activation have not been elucidated yet. Regarding the transduction signal, it has been demonstrated that the induction of CYP2E1 gene by IL4 in the B16A2 human hepatoma cell model does not involve the STAT6 pathway but rather the PKCζ pathway [7].

The present study was conducted in the HepG2 cell line and aims to determine the *cis*-acting element and the *trans*-acting factors involved in the induction of CYP2E1 expression by IL4. Moreover, our study attempts to determine the IL4 signal transduction pathway as well as the effect of IL1β on CYP2E1 promoter activity and to compare the *cis*-acting elements involved in both effects. Our results indicate that IL4 acts via 128-bp DNA sequence (−671/−544) on which several transcription factors can bind and mediate the transcriptional activation. The inhibition exerted by IL1β appears to require a different DNA fragment located between −982 and −811.

2. Materials and methods

2.1. Chemicals and cytokines

Human recombinant IL1β and IL4 were purchased from Tebu and R&DSystems, respectively. H7 (1-[5-isoquinolinesulfonyl]-2-methylpiperazine) was obtained from Sigma, PD98059 (2′-amino-3′-methoxyflavone), LY294002 [2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one] and rapamycin (Rapa) were purchased from Calbiochem.

2.2. Cell culture

The human hepatoma cell line HepG2 was cultured at 37 °C in an atmosphere of 5% CO2 in Dulbecco's modified eagle medium (DMEM)/HAM F12 (v/v) (Invitrogen) medium, supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen) and 0.5 μg/ml fungizone (Squibb). Medium was renewed every other day except after transfection and during cytokine treatment where it was changed daily.

2.3. Plasmids

The −1342 to +36 phCYP2E1-luc plasmid corresponds to a fragment of the human CYP2E1 gene promoter extending between −1342 to +36 placed in front of the firefly luciferase reporter gene as previously described [27]. The pα-glob-RL plasmid, which corresponds to the proximal promoter of the human α-globin gene placed in front of the *Renilla reniformis* luciferase gene, was previously utilized as a control of transfection efficiency [27] and is not sensitive to IL4 treatment. The various constructs

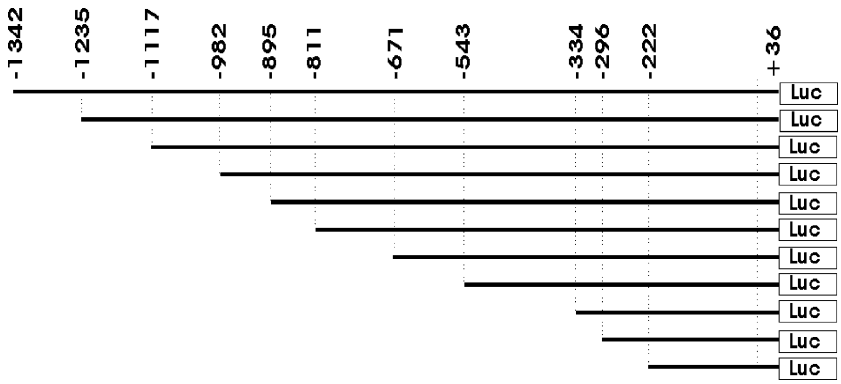

Fig. 1. Schematic representation of the phCYP2E1-luc constructs contained in pGL3. Numbers refer to the nucleotide position relative to the transcription start site. Each construct consists of the pGL3 plasmid carrying a fragment of the human CYP2E1 promoter (−1342/+36 for the longest one and −222/+36 for the shortest one) in front of the firefly luciferase reporter gene. The constructs were prepared as described in Section 2.

of the human CYP2E1 promoter differ from one another by sequential deletion of about 100-bp fragments from the 5′ end (Fig. 1). The −222 to +36 phCYP2E1-luc construct was prepared by digestion of the −1342 to +36 phCYP2E1-luc with *Nhe*I followed by ligation. The constructs −296 to +36 phCYP2E1-luc and −334 to +36 phCYP2E1-luc were prepared by digestion of the −1342 to +36 phCYP2E1-luc plasmid with *Kpn*I and *Sac*I, respectively, followed by ligation. The construct −543 to +36 phCYP2E1-luc was prepared by digestion of the −1342 to +36 phCYP2E1-luc plasmid with *Eco*RI and *Mlu*I, followed by blunt end generation and ligation. The remaining constructs were prepared by PCR amplification using the −1342 to +36 phCYP2E1-luc plasmid with a specific proximal primer complementary to −505/−523 and another distal one (complementary to −1235/−1226, −1117/−1099, −982/−962, −895/−877, −811/−792 or −671/−652). The six distal primers were ligated to *Xho*I linkers. The PCR products were digested with *Xho*I and *Eco*RI and inserted into the −1342 to +36 phCYP2E1-luc plasmid previously digested with the same couple of enzymes which led to removal of its distal promoter part. To obtain the heterologous constructs into the pTZ-TK-firefly luciferase plasmid (pTZ-TK-luc), the −671/−544 fragment of CYP2E1 promoter prepared by PCR was ligated to linkers containing *Bam*HI and *Kpn*I restriction sites. The (−671 to −544) pTZ-TK-luc was obtained by ligation of the PCR product and pTZ-TK-luc plasmid after their digestion with *Bam*HI and *Kpn*I. Various oligonucleotides of the −671/−544 fragment flanked by *Bam*HI and *Kpn*I restriction sites were designed: 1/128 5′GATCCTACAGCCAGAATATATACCTTTA AAAAAAATGAAAACAGAGAGGTAC3′, 2/128 5′GATCCTTAACTTTCTCAGAATTGGTT GACTCACTCTTTCCTTTTAGGTAC3′, 3/128 5′GATCCTTTTTCTTCCATGGAATTTTCCA GTTAACTTGAGAAAGTGGAATCGGTAC3′, 2 + 3/128 5′GATCCTTAACTTTCTCAGAATT GGTTGACTCACTCTTTCCTTTTATTTTTCTTCCATGGAATTTTCCAGTTAACTTGAGAA AGTGGAATCGGTAC3′ (Genset or Qiagen) and inserted into the pTZ-TK-luc plasmid. They gave rise, respectively, to the plasmids (−671/−629) pTZ-TK-luc, (−628/−589) pTZ-TK-luc, (−588/−544) pTZ-TK-luc and (-628/-544) pTZ-TK-luc. The constructs shown in Fig. 1 were controlled by restriction enzyme mapping and the constructs used in Fig. 5 were sequenced.

2.4. Transfection experiments

A day before transfection, HepG2 cells were seeded at a density of 45,000 cells/cm^2 into 24-well plates (10^5 cells per well). 0.6 μg of the plasmid tested and 0.06 μg of the pα-glob-RL control plasmid were introduced by the calcium phosphate co-precipitation technique. After 4 h of incubation with the precipitate, the medium was removed either directly by washing with phosphate buffer saline (PBS) or after a 2-min glycerol shock followed by washing with PBS. After an overnight incubation, the various agents were added for various times to the medium, which was changed daily. The cells were then homogenized in the passive lysis buffer (Promega) and luciferase activities were measured with the dual luciferase kit (Promega) in a luminometer. The pα-glob-RL was utilized to normalize transfection efficiency.

2.5. Nuclear protein extracts and gel shift assay

Nuclear extracts from HepG2 cells were prepared, protein concentration was determined and gel shift assays were performed as previously described [28] except that nuclear extracts were not dialyzed. Binding reactions were performed in 20 μl hepes buffer (20 mM) containing 0.15 mM EDTA, 50 mM NaCl, 65 mM KCl, 5 mM $MgCl_2$, 4 mM spermidine, 2.5 mM DTT, 100 Hg/ml bovine serum albumin, 15% glycerol and 1 μg poly-dIdC. The oligonucleotides used for the gel shift assays are the following: AP1 5′CTAGTGATGAGTCAGCCGGATC3′ [29], NFκB 5′AGTTGAGGGGACTTTCCCAGGCG3′ [30], STAT6 5′TAGTCAACTTCCCAAGAACAGA ATCA3′ [31], C/EBP 5′AAAGATGGTATGATTTTG-

TAATGGGGTAGGA3′ [32], NFAT 5′TC TAAGGAGGAAAAACTGTTTCATG3′ [33], 4/128 5′GGGCTCACTCTTTCCTTTTATTTTTC TTCCATGGAATTTT3′. The oligonucleotides 1/128, 2/128 and 3/128 have been described in the Plasmids section.

2.6. Statistical analysis

Statistical analyses were performed with Microsoft excel software by analyzing the variances and then applying the suitable Student's *t*-test.

3. Results

3.1. Dose-dependent response of CYP2E1 promoter to IL4 treatment

IL4 is known to induce the CYP2E1 apoprotein and its corresponding mRNA content in human hepatocytes as well as in B16A2 hepatoma cells. The increase of mRNA amount is, at least partially, due to transcription rate activation [7]. To determine whether IL4 enhances CYP2E1 promoter activity, we conducted a dose–response analysis. Cells were transfected with the largest CYP2E1 promoter construct −1342 to +36 phCYP2E1-luc and the control plasmid pα-glob-RL, treated or not with IL4 (dose range from 0.08 to 50 ng/ml) for 48 h and harvested for luciferase activities assessment. CYP2E1 promoter activity was slightly induced (1.3-fold) by 0.2 ng/ml IL4 and maximal induction level (two to three-fold) was reached with IL4 concentrations between 2 and 10 ng/ml (Fig. 2). This result demonstrates that the human CYP2E1 promoter sequence between −1342 and +36 contains all the *cis*-acting elements required for induction by IL4 and that maximal induction level is reached for the concentration 2 ng/ml. Time course analysis showed that the IL4 effect was already maximal after a 24 h-treatment but that it decreased after 72 h (data not shown).

3.2. Identification of the IL4-responsive region in CYP2E1 promoter

In order to locate the IL4-responsive region in the CYP2E1 promoter sequence, serially truncated fragments of the −1342/+36 promoter region were linked to the firefly luciferase reporter gene and the constructs were transfected in HepG2 cells together with the pα-glob-RL normalization vector. After transfection, the cells were treated with 10 ng/ml IL4 for 48 h and luciferase activity was measured (Fig. 3). Progressive deletion of the sequence upstream of −672 tended to slightly decrease the effect of IL4, but this decrease was not statistically significant. When the fragment −671/−544 was deleted, the promoter lost its responsiveness to IL4. Indeed, luciferase activity of the construct (−543/+36) in the presence of IL4 was 105% that of the control untreated cells ($n = 12$, $P < 0.0001$). Similarly, the three shortest constructs, −334 to +36 phCYP2E1-luc, −296 to +36 phCYP2E1-luc and −222 to +36 phCYP2E1-luc were not responsive to IL4 treatment. These results clearly demonstrate that the induction by IL4 of the CYP2E1 gene transcription is, at least partially, mediated by a 128-bp sequence extending between −671 and −544. It is possible that upstream sequences could also be involved in this regulation but their contribution appears to be moderate at best.

3.3. Putative transcription factor binding sites in the IL4-responsive region

In order to analyze the IL4-responsive region in CYP2E1 promoter, we performed a MatInspector profes-

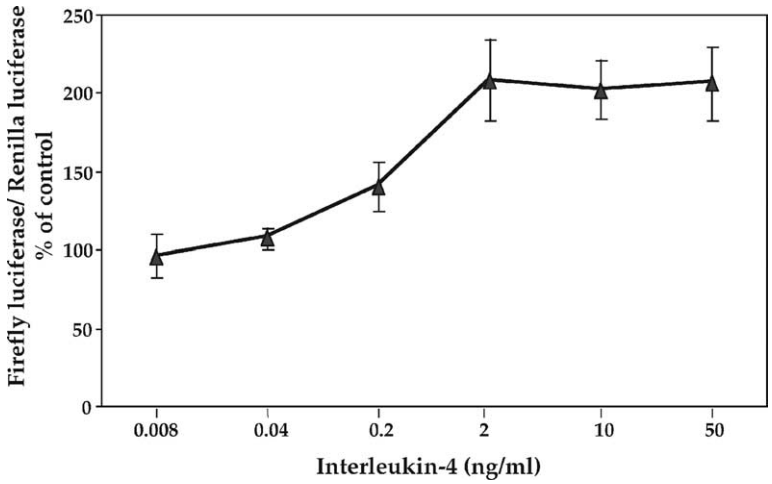

Fig. 2. Dose–response analysis of IL4 effect on −1342 to +36 phCYP2E1-luc transiently expressed in HepG2 cells. For each measurement, 10^5 cells were co-transfected with a precipitate of 0.6 μg −1342 to +36 phCYP2E1-luc plasmid and 0.06 μg pα-glob-RL control plasmid. Twenty hours later, the cells were treated or not with different doses of IL4 for 48 h and the luciferase activities were measured. Results, expressed as percent of the control untreated cells, represent the mean ± S.D. of three independent transfections.

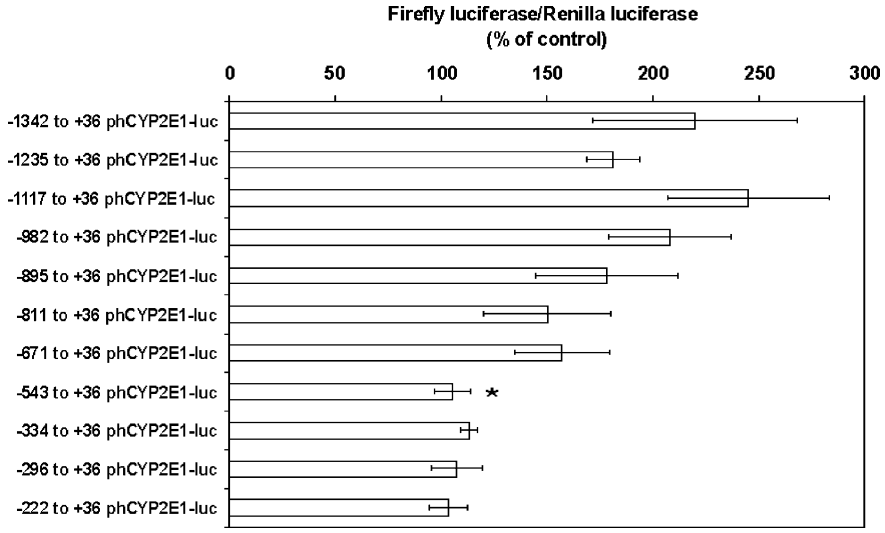

Fig. 3. Identification of the IL4-responsive region in the CYP2E1 gene promoter. For each measurement, 10^5 HepG2 cells were co-transfected with a precipitate of 0.6 μg of each phCYP2E1-luc construct and 0.06 μg pα-glob-RL control plasmid. Twenty hours later, the cells were treated or not with 10 ng/ml IL4 for 48 h and the luciferase activities were measured. Results are expressed as percent of the corresponding control untreated cells and represent the mean ± S.D. of 3–16 independent transfection experiments, each performed in triplicate or quadruplicate. Statistically significant difference ($^*P < 0.0001$) relative to activity of the −671 to +36 phCYP2E1-luc construct.

sional analysis (www.genomatix.de/mat_fam) of the −671/−544 sequence. Several putative binding sites for transcription factors (STAT5, activator protein (AP1), nuclear factor of activated T cells (NFAT), STAT6, nuclear factor kappa-B (NFκB) and CCAAT enhancer binding protein (C/EBPβ)) were found in the 128 bp DNA sequence which confers responsiveness to IL4 (Fig. 4). Most of them are known for their involvement in IL4 response in other cell systems.

3.4. Responsiveness of the heterologous promoter pTZ-TK to IL4 after insertion of the IL4-responsive region

In order to determine whether the −671/−544 CYP2E1 promoter sequence was sufficient to confer responsiveness to IL4, the whole region or several fragments were inserted upstream the firefly luciferase reporter gene driven by the thymidine kinase (TK) promoter (pTZ-TK-luc). The 128 bp-sequence was divided into three fragments depending on the location of putative transcription factor binding sites (Fig. 4). The sequences −671 to −629 (1/128), −628 to −589 (2/128) and −588 to −12 544 (3/128) and a fragment corresponding to −628 to −544 (fragment 2+3/128) were tested. These constructs were co-transfected with pα-glob-RL normalization vector in HepG2 cells which were treated or not with 10 ng/ml IL4 for 48 h. Luciferase activity was then measured (Fig. 5). The whole 128 bp sequence mediated a potent induction of the heterologous promoter (2.6-fold induction, $P < 0.0001$) whereas the three oligonucleotides 1/128, 2/128 and 3/128 were not able to confer IL4 responsiveness when inserted independently. However, the fragment 2 + 3/128 conferred a partial but significant induction by IL4 (1.7-fold induction, $P < 0.05$). These results clearly show that full CYP2E1 gene induction by IL4 requires the complete −671/

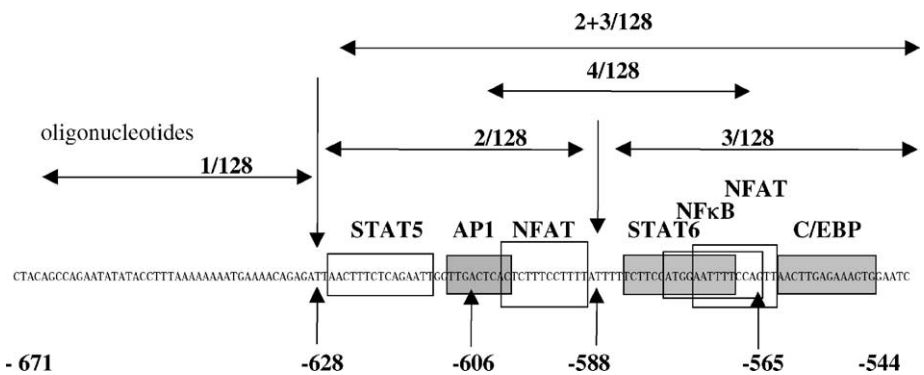

Fig. 4. Configuration of the IL4-reponse region extending from −671 to −544. MatInspector professional analysis was performed. Arrows and the associated numbers refer to names and positions of the oligonucleotides used in some constructs and in the gel-shift experiments.

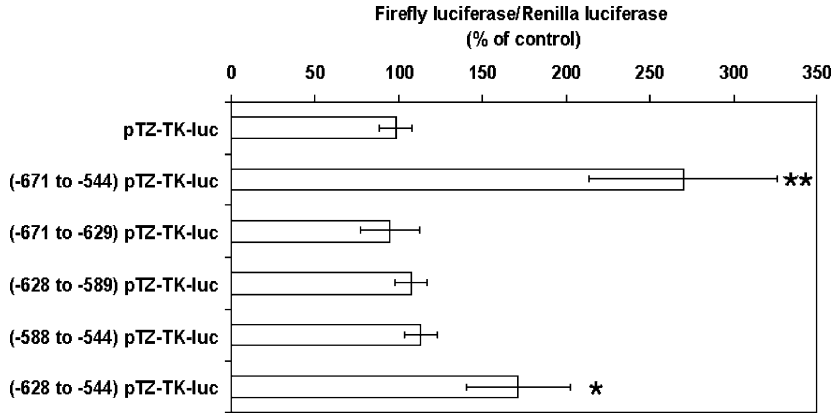

Fig. 5. Effect of IL4 on pTZ-TK-luc containing fragments of the IL4-response region. The −671/−544 CYP2E1 promoter region or fragments of this sequence were inserted in front of pTZ-TK-luc. For each measurement, 10^5 HepG2 cells were co-transfected with a precipitate of 0.6 μg of each pTZ-TK-luc construct and 0.06 μg pα-glob-RL control plasmid. Twenty hours later, the cells were treated or not with 10 ng/ml IL4 for 48 h and the luciferase activities were measured. Values, expressed as percent of the corresponding control untreated cells, represent the mean ± S.D. of 4–14 independent transfection experiments, each performed in quadruplicate. Statistically significant difference ($^*P < 0.05$, and $^{**}P < 0.0001$, respectively) with respect to pTZ-TK-luc activity.

−544 sequence and that the putative STAT6 binding site is not sufficient to mediate the IL4 effect.

3.5. Effect of IL1β alone or combined with IL4 on the CYP2E1 promoter activity

IL1β is known to repress CYP2E1 expression in different models in vivo and in vitro. However, its transcriptional effect on CYP2E1 gene has not yet been elucidated. In order to investigate IL1β effect on CYP2E1 promoter activity and to study the effect of a combination between inflammatory and anti-inflammatory cytokines, we treated HepG2 cells transfected with the −1342 to +36 phCYP2E1-luc with 10 ng/ml IL1β and/or 10 ng/ml IL4 for 48 h. IL1β treatment caused a small but significant decrease of CYP2E1 promoter activity (17%, $P < 0.05$, $n = 12$) whereas IL4 induced a 2.2-fold increase of the promoter activity (Fig. 6A). The combination of the two cytokines led to an induction level similar to that of IL4 alone, showing that the positive IL4 effect is dominant.

In an attempt to locate the CYP2E1 promoter sequence that mediates the inhibition by IL1β, we treated HepG2 cells transfected with various CYP2E1 promoter constructs with 10 ng/ml IL1β for 48 h prior to luciferase activity assessment. Results are expressed as percent of firefly luciferase activity of the corresponding untreated cells (Fig. 6B). The inhibition exerted by IL1β required the promoter fragment between −982 and −811 since its deletion abolished completely the effect. The specificity of IL1β effect was further supported by the absence of repression of the control pTZ-TK promoter, which was in fact slightly increased by the cytokine. This result demon-

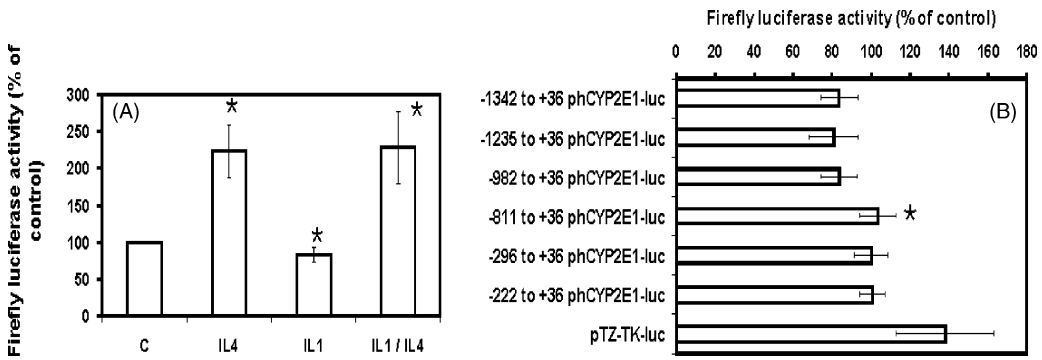

Fig. 6. (A) Effect of IL1β and IL4 on the -1342 to +36 phCYP2E1 promoter activity transiently transfected into HepG2 cells. For each measurement, 10^5 cells were transfected with a precipitate of 0.6 μg −1342/+36 phCYP2E1-luc plasmid. Twenty hours later, the cells were treated or not with 10 ng/ml IL1β and/or 10 ng/ml IL4 for 48 h and the luciferase activity was measured. Results, expressed as percent of the corresponding control untreated cells, represent the mean ± S.D. of 6–16 independent transfection experiments, each performed in triplicate or quadruplicate. (B) Effect of IL1β on CYP2E1 promoter constructs transiently transfected into HepG2 cell line. For each measurement, 10^5 cells were transfected with a precipitate of 0.6 μg of each phCYP2E1-luc construct or pTZ-TK-luc plasmid. Twenty hours later, the cells were treated or not with 10 ng/ml IL1β for 48 h and luciferase activity was measured. Values, expressed as percent of the corresponding control untreated cells, represent the mean ± S.D. of 5–12 independent transfection experiments, each performed in quadruplicate. Statistically significant difference ($^*P < 0.001$) with respect to the control untreated cells (part A) or to the −982 to +36 phCYP2E1-luc construct (part B).

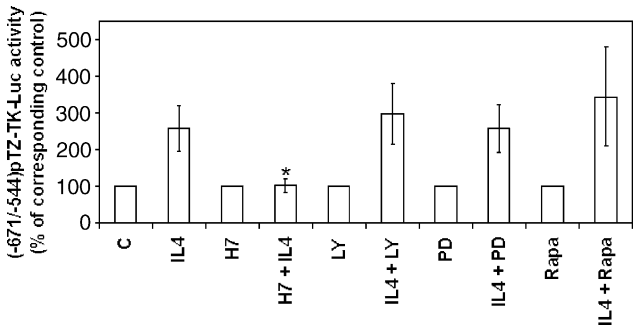

Fig. 7. Effect of signal transduction inhibitors on induction by IL4. For each measurement, 10^5 HepG2 cells were co-transfected with a precipitate of 0.6 μg of (−671/−544) pTZ-TK-luc and 0.06 μg pα-glob-RL control plasmid. Twenty hours later, the cells were simultaneously treated with 10 ng/ml IL4 and/or 50 μM H7, 200 nM rapamycin (Rapa), 50 μM LY294002 (LY) or 50 μM PD98059 (PD) for 48 h and luciferase activities were measured. C refers to the control untreated cells. Values correspond to normalized promoter activity (firefly luciferase/renilla luciferase) and are expressed as percent of the corresponding control untreated cells. They represent the mean ± S.D. of two or three independent transfection experiments, each performed in quadruplicate. Statistically significant difference with respect to activity of cells treated by IL4 alone ($^*P < 0.01$).

strates that IL1β acts via a sequence different from the IL4-responsive region and is in accordance with the absence of interaction between these two cytokines.

3.6. Effect of signal transduction inhibitors on the IL4 effect

In an attempt to uncover the transduction pathway involved in IL4-induced CYP2E1 promoter activity, we treated HepG2 cells co-transfected with the (−671/−544) pTZ-TK-luc plasmid and the pα-glob-RL normalization vector simultaneously with IL4 and various signal transduction inhibitors that have been reported to be involved in cytokine transduction pathways. After 48 h of treatment, luciferase activities were measured (Fig. 7). These experiments showed that H7, a PKC inhibitor, blocked completely the effect of IL4 on (−671/−544) pTZ-TK-luc (Fig. 7). LY294002, a PI3 kinase inhibitor, and PD98059, a MAP kinase inhibitor, failed to block the IL4-induced promoter activity. The lack of effect was also observed when the inhibitors were added to the culture medium 30 min before cytokine addition (data not shown). Rapamycin, an inhibitor of p70S6 kinase, was also ineffective. These results demonstrate that the IL4 effect, in our experimental conditions, is independent of MAP kinase, PI3 kinase and p70S6 kinase and requires PKC activation.

3.7. Nuclear protein binding to the (−671/−544) IL4-responsive region

Gel mobility shift assays were conducted to determine whether the IL4-responsive region of the CYP2E1 promoter could specifically interact with nuclear proteins. The various probes 1/128, 2/128, 3/128 and 4/128 (see Fig. 4) were labeled with ^{32}P and incubated with nuclear extracts

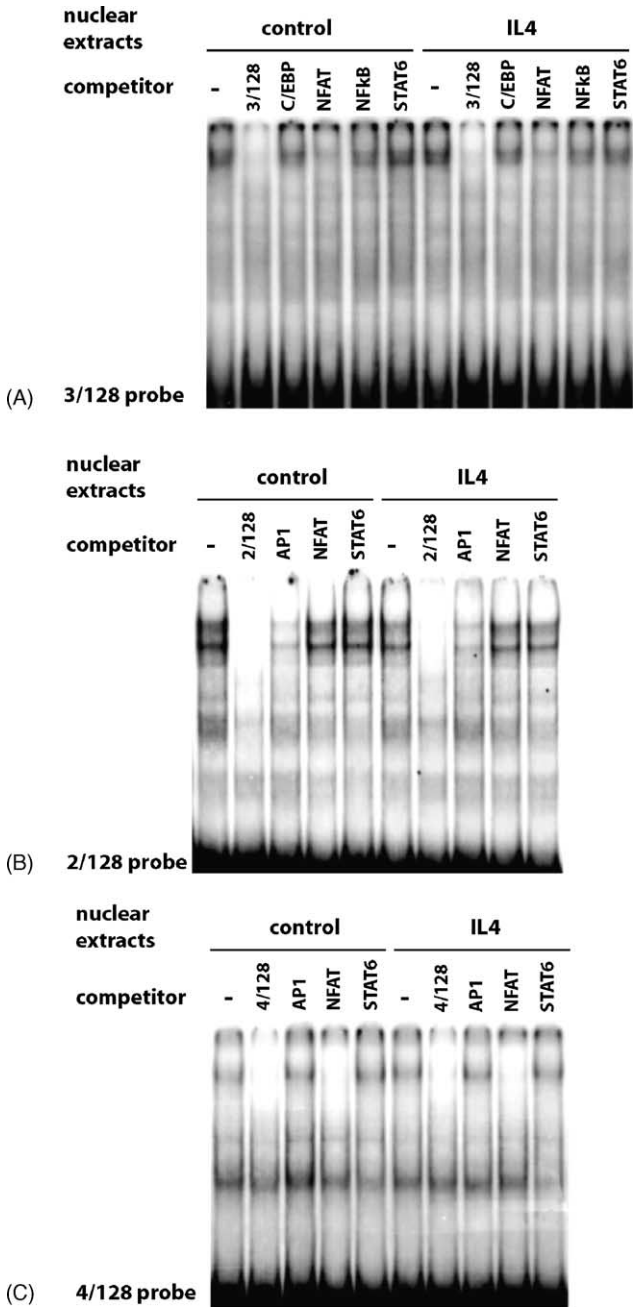

Fig. 8. Gel shift assays. Labeled probe 3/128 (A), 2/128 (B) or 4/128 (C) was incubated with 8 μg nuclear extracts from HepG2 cells treated or not with IL4 for 1 h. In the indicated lanes, a 100-fold molar excess of unlabeled competitor was added to the binding reaction. The gel shift assays were reproduced in two independent experiments with different cell extracts.

prepared from IL4-treated (1 h) and untreated cells. Competition experiments using a 100-fold excess of consensus oligonucleotides for the transcription factors which may bind to the putative sites in the CYP2E1 promoter region (see Fig. 4) were carried out. Nucleoprotein complexes were observed with probe 1/128 but no consensus sequence for a known transcription factor was found in this sequence (data not shown). The 3/128 probe formed one major complex with HepG2 nuclear extracts, which was competed by itself and by the unlabeled NFAT oligonucleotide

(Fig. 8A). In contrast, neither C/EBP nor NFκB or STAT6 consensus oligonucleotides displaced the complex (Fig. 8A). Two major complexes were obtained with the 2/128 probe, which disappeared when a 100-fold excess of cold probe was added to the binding reaction (Fig. 8B). Competition experiments showed that an excess of the AP1 oligonucleotide competed with the 2/128 fragment whereas neither NFAT nor STAT6 consensus sequences displaced the retarded bands (Fig. 8B). The 4/128 probe, overlapping the region between probes 2/128 and 3/128 (see Fig. 4) was also tested. A specific band was observed, which was competed by both the cold 4/128 probe and the NFAT consensus oligonucleotide (Fig. 8C). Although the NFAT binding site revealed by probe 4/128 was also included into the 2/128 sequence, it was probably too close to the end of the 2/128 probe to bind efficiently nuclear proteins. None of the various complexes was affected by the 1 h IL4 treatment. These results suggest that AP1 and NFAT transcription factors bind to three sites in the (−671/−544) IL4-responsive sequence.

4. Discussion

CYP2E1 plays a key role in toxicology, yet its transcriptional regulation is still poorly understood. Its induction by IL4 and its repression by inflammatory cytokines are of particular importance since these cytokines play key roles during immune response and liver diseases. The goal of the present study was to determine the *cis*-acting element(s) and the *trans*-acting factor(s) as well as the transduction pathway(s) which are involved in CYP2E1 induction by IL4. In addition, we investigated the effect of IL1β and compared it with that of IL4 in terms of sequences mediating its inhibitory effect on CYP2E1 promoter. Our results provide clear evidences that IL4-induced CYP2E1 promoter activity depends on a 128 bp sequence (−671/−544) which contains several putative binding sites for transcription factors. At least an AP1-like factor and a NFAT-like factor are able to bind to this IL4-responsive region. Moreover, we provide evidence that the PKC transduction pathway mediates the IL4-induced CYP2E1 promoter activity in HepG2 cells. IL1β represses slightly but significantly the transfected CYP2E1 promoter activity, thereby demonstrating that IL1β-mediated inhibition of the CYP2E1 gene expression occurs at least partially at the transcriptional level. We found that the IL1β-responsive sequence is distinct from the IL4-responsive region and is located between −982 and −811. When the two cytokines were combined, the IL4 inducing effect prevailed over the IL1β inhibitory effect.

We conducted the experiments on HepG2 cells, which are responsive to many cytokines such as IL1β, IL4 and IL6 [34–37] and are known to express proteins required for IL4 signal transduction [38]. Furthermore, HepG2 cells show basal and induced expression of certain CYP genes [34] but lack basal or inducible CYP2E1 activity. In fact, IL4 as well as ethanol treatment failed to induce the endogenous CYP2E1 gene (data not shown). Nevertheless, the use of such a cell line is justified by the fact that the cells are responsive to IL4 [37] and indeed, IL4 induced the transfected CYP2E1 promoter activity (the present work). Moreover, HepG2 cells have been utilized for heterologous expression of the rabbit CYP2E1 gene [2]. Altogether, these data suggest that absence of CYP2E1 gene expression in HepG2 cell line is not due to the lack of appropriate regulatory *trans*-acting factors, but rather to the repression of the endogenous gene locus.

Our finding of a complex IL4-responsive region is not surprising since a 101 bp IL4-responsive region has been characterized in the murine CD23-encoding gene [39] and a 198 bp sequence encompassing STAT6 and C/EBP binding sites, forms the IL4-responsive region in the polymeric Ig receptor gene [26]. Requirement of such a long DNA stretch to mediate IL4 effect seems intriguing since IL4 immunological effects are well known to rely on the JaK1-STAT6 pathway which is critical as demonstrated by several reports on animals lacking normal STAT6 gene [40–42]. Such animals are unable to develop the IL4-related aspects of a normal immune response [43]. However, the JaK1-STAT6 pathway is in fact common to other cytokine signaling such as IL3 and platelet-derived growth factor [44], thereby suggesting that other transduction pathways and transcription factors could be required to elicit cytokine-specific responses. In fact, although the STAT6 binding site is sufficient to mediate induction of certain genes by IL4 [31], many recent studies demonstrated that STAT6 is not the only transducer and that IL4 signal transduction occurs via various pathways which are species-, cell type- and also receptor-specific [12,18–20]. Our results with the (−671/−544) pTZ-TK-luc plasmid and the gel shift assays provide clear evidence that induction of CYP2E1 gene expression by IL4 requires more than the STAT6 binding site and is mediated by PKC and not by MAP kinase, PI3 kinase or p70S6 kinase. These results agree with those of Lagadic-Gossmann et al. [7] suggesting the involvement of the in IL4-mediated CYP2E1 induction. PKC was reported to be involved in the regulation by IL4 of several target genes [22,23,45].Thus, STAT6, despite its importance, seems to be insufficient to mediate all activator effects elicited by IL4. This transcription factor is already known to interact with other transcription factors such as c-Jun, SP1, C/EBP and NFκB in order to affect gene expression (review by [12]). Most of the recent studies demonstrated that IL4 mediates its stimulatory effect through recruitment of *trans*-acting factors which collaborate or not with STAT6 [26]. Lagadic-Gossmann et al. [7] clearly showed that STAT6, although phosphorylated in response to IL4 treatment, is not involved in IL4-induced CYP2E1 expression. In human lymphoblastoid cells, the IL4-induced complement receptor-2 promoter activity involves AP1 and C/EBP [46], apparently without

STAT6 intervention. The IL4-responsive region of the murine CD23 promoter encompasses STAT6 and NFκB binding sites which are both required for stimulation by IL4 [47]. Other studies on the human Ig heavy chain Sγ3 region and the IgE germline promoter showed cooperation between proteins binding to NFκB/Rel and STAT6 motifs to mediate induction by IL4 [48,49]. Also the STAT6-mediated induction of 15-lipoxygenase-1 gene by IL4 requires increased acetylation activity of CBP/p300 [50].

Schaffer [49] showed also the presence of a putative C/EBP binding site in the IL4-responsive region of the Ig heavy chain Sγ3. C/EBP has been shown to interact with STAT6 and other transcription factors on the IL4-response region in immunoglobulin heavy chain germline ε promoter [51]. Cooperation between STAT6 and C/EBP has been reported for the IL4-mediated induction of germline ε transcripts before class switching [52]. Indeed, C/EBPβ is thought to stabilize STAT6 at its binding site [53]. Our results with the IL4-responsive region inserted in the pTZ-TK heterologous promoter are in agreement with previous studies. The STAT6 motif inserted alone in the TK promoter does not confer responsiveness to IL4 in HepG2 and U-937 cells [54]. Insertion of either a NFκB binding site [48] or a C/EBP binding site [55] together with the STAT6 binding site in a heterologous promoter has been shown to be necessary in order to confer responsiveness to IL4. In agreement with these papers, our findings showed that the fragment 3/128 (bearing STAT6 and other putative binding sites) is not sufficient to mediate induction by IL4. Furthermore, a fragment of the IL4-responsive region overlapping both the 2/128 and 3/128 sequences, but lacking the C/EBP and STAT5 binding sites, inserted in front of the heterologous TK promoter failed to confer responsiveness to IL4 (data not shown). Indeed, a mechanism involving cooperation between STAT6, C/EBPβ and other transcription factors was reported for the IL4-mediated induction of germline ε promoter activity [53].

Using electrophoretic mobility shift assays, we also found that nuclear proteins bind to the fragment (−671/−544) responsible for the increased transcription of the promoter by IL4. Competition assays with consensus oligonucleotides suggest that the NFAT and AP1 nucleoproteins bind to three sites in this region. We observed no modification in the intensity of the various retarded bands when the cells were treated by IL4. Although several studies have reported increased binding activity of transcription factors to regulatory sequences (STAT6, AP1, cAMP response element-binding protein (CREB)) following IL4 treatment [44,46], other authors have reported no modification in the binding of transcription factors (AP1, Oct1, NFκB) after IL4 treatment [49,56,57]. NFAT is activated by Ca^{++} in response to the release of inositol triphosphate by phospholipase C, which also generates diacyl glycerol [58]. Ca^{++}-dependent NFAT activation is mediated by the calcineurin dephosphorylating activity. Diacyl glycerol, together with Ca^{++}, is also required for activation of certain PKC forms. Therefore, coordination between NFAT and targets of PKC could occur in our system. Binding activity of AP1, a well known target of PKC, is induced by IL4 in cultured human hepatocytes [6] and AP1 is involved in the IL4-induced complement receptor-2 promoter activity [46]. Therefore, in addition to a putative role for NFAT, a contribution of AP1 to the IL4-mediated CYP2E1 induction is likely. A cooperation among various IL4 signal transduction pathways is probably involved in our system and was reported for other targets such as the 3-β-hydroxysteroid dehydrogenase gene [59].

With respect to IL1β, which displays a tissue- and species-specific effect on CYP2E1 expression, it caused a small inhibition of CYP2E1 promoter activity. Therefore, the IL1β inhibitory effect is at least in part caused by a decrease of transcription activity via a −982/−811 sequence, which is independent of the IL4-responsive region. A recent report showed that the rat CYP2E1 promoter activity is still inhibited, in hepatoma cell lines, by IL1β through a minimal promoter fragment between −40 and +28 whereas IL6 inhibits this promoter activity via the −669/−507 sequence [8]. Discrepancy between these reports and our findings is probably due to species-specific differences.

IL4 is known to interact with inflammatory cytokines such as ILβ and IL6 in various cell models. For instance, IL4 counteracts the effect of IL1 and IL6 on certain acute phase proteins [37,60], thereby confirming its anti-inflammatory property [15]. Accordingly, IL4 enhances the IL1β-induced expression of IL1 receptor antagonist [37]. Our results demonstrated that IL4 effect masks the small IL1β inhibitory effect and no interaction seems to occur between those cytokines, probably because the target regulatory sequences in the CYP2E1 promoter are distinct.

In conclusion, this study demonstrated that induction of CYP2E1 by IL4 requires a 128bp sequence where several transcription factors are likely to bind. The PKC transduction pathway mediates the IL4 effect in our model. IL1β slightly inhibits CYP2E1 promoter activity through a sequence independent from the IL4-responsive region.

Acknowledgments

This work was supported by the INSERM, the Université Paris V René Descartes, the Région Ile de France, the Ligue Nationale contre le Cancer and the ATC INSERM Alcool.

References

[1] Gonzalez FJ, Ueno T, Umeno M, Song BJ, Veech RL, Gelboin HV. Microsomal ethanol oxidizing system: transcriptional and post-transcriptional regulation of cytochrome P450 CYP2E1. Alcohol Alcohol Suppl 1991;1:97–101.

[2] Peng HM, Coon MJ. Regulation of rabbit cytochrome P450 2E1 expression in HepG2 cells by insulin and thyroid hormone. Mol Pharmacol 1998;54:740–7.
[3] Song BJ, Gelboin HV, Park SS, Yang CS, Gonzalez FJ. Complementary DNA and protein sequences of ethanol-inducible rat and human cytochrome P-450s. Transcriptional and post transcriptional regulation of the rat enzyme. J Biol Chem 1986;261:16689–97 [Erratum in: J Biol Chem 1987;262:8940].
[4] Umeno M, McBride OW, Yang CS, Gelboin HV, Gonzalez FJ. Human ethanol-inducible P450IIE1: complete gene sequence, promoter characterization, chromosome mapping, and cDNA-directed expression. Biochemistry 1988;27:9006–13.
[5] Abdel-Razzak Z, Loyer P, Fautrel A, Gautier JC, Corcos L, Turlin B, et al. Cytokines down-regulate expression of major cytochrome P-450 enzymes in adult human hepatocytes in primary culture. Mol Pharmacol 1993;44:707–15.
[6] Langouet S, Corcos L, Abdel-Razzak Z, Loyer P, Ketterer B, Guillouzo A. Up-regulation of glutathione *S*-transferases alpha by interleukin-4 in human hepatocytes in primary culture. Biochem Biophys Res Commun 1995;216:793–800.
[7] Lagadic-Gossmann D, Lerche C, Rissel M, Joannard F, Galisteo M, Guillouzo A, et al., The induction of the human hepatic CYP2E1 gene by interleukin 4 is transcriptional and regulated by protein kinase C. Cell Biol Toxicol 2000;16:221–33.
[8] Hakkola J, Hu Y, Ingelman-Sundberg M. Mechanisms of down-regulation of CYP2E1 expression by inflammatory cytokines in rat hepatoma cells. J Pharmacol Exp Ther 2003;304:1048–54.
[9] Peng HM, Coon MJ. Promoter function and the role of cytokines in the transcriptional regulation of rabbit CYP2E1 and CYP2E2. Arch Biochem Biophys 2000;382:129–37.
[10] Tindberg N, Baldwin HA, Cross AJ, Ingelman-Sundberg M. Induction of cytochrome P450 2E1 expression in rat and gerbil astrocytes by inflammatory factors and ischemic injury. Mol Pharmacol 1996;50:1065–72.
[11] Hu Y, Hakkola J, Oscarson M, Ingelman-Sundberg M. Structural and functional characterization of the 5′-flanking region of the rat and human cytochrome P450 2E1 genes: identification of a polymorphic repeat in the human gene. Biochem Biophys Res Commun 1999;263:286–93.
[12] Nelms K, Keegan AD, Zamorano J, Ryan JJ, Paul WE. The IL-4 receptor: signaling mechanisms and biologic functions. Annu Rev Immunol 1999;17:701–38.
[13] Banchereau J, Defrance T, Galizzi JP, Miossec P, Rousset F. Human interleukin 4. Bull Cancer 1991;78:299–306.
[14] Gautam SC, Chikkala NF, Hamilton TA. Anti-inflammatory action of IL-4. Negative regulation of contact sensitivity to trinitrochlorobenzene. J Immunol 1992;148:1411–5.
[15] Vannier E, Miller LC, Dinarello CA. Coordinated antiinflammatory effects of interleukin 4: interleukin 4 suppresses interleukin 1 production but up-regulates gene expression and synthesis of interleukin 1 receptor antagonist. Proc Natl Acad Sci USA 1992;89:4076–80.
[16] Callard RE, Matthews DJ, Hibbert I. IL-4 and IL-13 receptors: are they one and the same? Immunol Today 1996;17:108–10.
[17] Idzerda RL, March CJ, Mosley B, Lyman SD, Vanden Bos T, Gimpel SD, et al. Human interleukin 4 receptor confers biological responsiveness and defines a novel receptor superfamily. J Exp Med 1990;171:861–73.
[18] David M, Ford D, Bertoglio J, Maizel AL, Pierre J. Induction of the IL-13 receptor alpha2-chain by IL-4 and IL-13 in human keratinocytes: involvement of STAT6 ERK and p38 MAPK pathways. Oncogene 2001;20:6660–8.
[19] Doucet C, Jasmin C, Azzarone B. Unusual interleukin-4 and -13 signaling in human normal and tumor lung fibroblasts. Oncogene 2000;19:5898–905.
[20] Finney M, Guy GR, Michell RH, Gordon J, Dugas B, Rigley KP, et al. Interleukin 4 activates human B lymphocytes via transient inositol lipid hydrolysis and delayed cyclic adenosine monophosphate generation. Eur J Immunol 1990;20:151–6.
[21] Lee YW, Kuhn H, Hennig B, Neish AS, Toborek M. IL-4-induced oxidative stress upregulates VCAM-1 gene expression in human endothelial cells. J Mol Cell Cardiol 2001;33:83–94.
[22] Ikizawa K, Kajiwara K, Basaki Y, Koshio T, Yanagihara Y. Evidence for a role of phosphatidylinositol 3-kinase in IL-4-induced germline C epsilon transcription. Cell Immunol 1996;170:134–40.
[23] Yanagihara Y, Basaki Y, Ikizawa K, Kajiwara K. Possible role of nuclear factor-kappa B activity in germline C epsilon transcription in a human Burkitt lymphoma B cell line. Cell Immunol 1997;176:66–74.
[24] Guzdek A, Stalinska K, Guzil K, Koj A. Differential responses of hematopoietic and non-hematopoietic cells to anti-inflammatory cytokines: IL-4 IL-13, and IL-10. J Physiol Pharmacol 2000;51:387–99.
[25] Hoeck J, Woisetschlager M. STAT6 mediates eotaxin-1 expression in IL-4 or TNF-alpha-induced fibroblasts. J Immunol 2001;166: 4507–15.
[26] Schj erven H, Brandtzaeg P, Johansen FE. Mechanism of IL-4-mediated up-regulation of the polymeric Ig receptor: role of STAT6 in cell type-specific delayed transcriptional response. J Immunol 2000;165:3898–906.
[27] Morel Y, de Waziers I, Barouki R. A repressive cross-regulation between catalytic and promoter activities of the CYP1A1 and CYP2E1 genes: role of H2O2. Mol Pharmacol 2000;57:1158–64.
[28] Cereghini S, Blumenfeld M, Yaniv M. A liver-specific factor essential for albumin transcription differs between differentiated and dedifferentiated rat hepatoma cells. Genes Dev 1988;2:957–74.
[29] Li Y, Jaiswal AK. Regulation of human NAD(P)H: quinone oxidoreductase gene. Role of AP1 binding site contained within human antioxidant response element. J Biol Chem 1992;267:15097-104 [Erratum in: J Biol Chem 1993;268:21454].
[30] Nakao S, Ogtata Y, Shimizu E, Yamazaki M, Furuyama S, Sugiya H. Tumor necrosis factor alpha-induced prostaglandin E2 release is mediated by the activation of cyclooxygenase-2 transcription via NFkB in human gingival fibroblasts. Mol Cell Biochem 2002;238: 11–8.
[31] Kraus J, Borner C, Giannini E, Hickfang K, Braun H, Mayer P, et al. Regulation of mu-opioid receptor gene transcription by interleukin-4 and influence of an allelic variation within a STAT6 transcription factor binding site. J Biol Chem 2001;276:43901–8.
[32] Garlatti M, Tchesnokov V, Daheshia M, Feilleux-Duche S, Hanoune J, Aggerbeck M, et al. CCAAT/enhancer-binding protein-related proteins bind to the unusual promoter of the aspartate aminotransferase housekeeping gene. J Biol Chem 1993;268:6567–74.
[33] Yaseen NR, Maizel AL, Wang F, Sharma S. Comparative analysis of NFAT (nuclear factor of activated T cells) complex in human T and B lymphocytes. J Biol Chem 1993;268:14285–93.
[34] Fukuda Y, Ishida N, Noguchi T, Kappas A, Sassa S. Interleukin-6 down regulates the expression of transcripts encoding cytochrome P450 IA1 IA2 and IIIA3 in human hepatoma cells. Biochem Biophys Res Commun 1992;184:960–5.
[35] Fukuda Y, Sassa S. Suppression of cytochrome P450IA1 by interleukin-6 in human HepG2 hepatoma cells. Biochem Pharmacol 1994;47:1187–95.
[36] Doostdar H, Grant MH, Melvin WT, Wolf CR, Burke MD. The effects of inducing agents on cytochrome P450 and UDP-glucuronyltransferase activities in human HEPG2 hepatoma cells. Biochem Pharmacol 1993;46:629–35.
[37] Gabay C, Porter B, Guenette D, Billir B, Arend WP. Interleukin-4 and IL-13 enhance the effect of IL-1beta on production of IL-1 receptor antagonist by human primary hepatocytes and hepatoma HepG2 cells: differential effect on C-reactive protein production. Blood 1999;93:1299–307.
[38] Verbsky JW, Bach EA, Fang YF, Yang L, Randolph DA, Fields LE. Expression of Janus kinase 3 in human endothelial and other non-lymphoid and non-myeloid cells. J Biol Chem 1996;271:13976–80.

[39] Richards ML, Katz DH. Regulation of the murine Fc epsilon RII (CD23) gene. Functional characterization of an IL-4 enhancer element. J Immunol 1994;152:3453–66.
[40] Takeda K, Tanaka T, Shi W, Matsumoto M, Minami M, Kashiwamura S, et al. Essential role of Stat6 in IL-4 signalling. Nature 1996; 380:627–30.
[41] Kaplan MH, Schindler U, Smiley ST, Grusby MJ. Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. Immunity 1996;4:313–9.
[42] Shimoda K, van Deursen J, Sangster MY, Sarawar SR, Carson RT, Tripp RA, et al. Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. Nature 1996;380:630–3.
[43] O'Shea JJ, Gadina M, Schreiber RD. Cytokine signaling in 2002: new surprises in the Jak/Stat pathway. Cell 2002;109:S121–31.
[44] Patel BK, Wang LM, Lee CC, Taylor WG, Pierce JH, LaRochelle WJ. Stat-6 and Jak1 are common elements in platelet-derived growth factor and interleukin-4 signal transduction pathways in NIH 3T3 fibroblasts. J Biol Chem 1996;271:22175–82.
[45] Shubinsky G, Schlesinger M. The mechanism of interleukin 4-induced down-regulation of CD38 on human B cells. Cell Immunol 1996;173:87–95.
[46] Vereshchagina LA, Tolnay M, Tsokos GC. Multiple transcription factors regulate the inducible expression of the human complement receptor 2 promoter. J Immunol 2001;166:6156–63.
[47] Richards ML, Katz DH. Analysis of the promoter elements necessary for IL-4 and anti-CD40 antibody induction of murine Fc epsilon RII (CD23): comparison with the germline epsilon promoter. J Immunol 1997;158:263–72.
[48] Messner B, Stutz AM, Albrecht B, Peiritsch S, Woisetschlager M. Cooperation of binding sites for STAT6 and NF kappa B/rel in the IL-4-induced up-regulation of the human IgE germline promoter. J Immunol 1997;159:3330–7.
[49] Schaffer A, Cerutti A, Shah S, Zan H, Casali P. The evolutionarily conserved sequence upstream of the human Ig heavy chain S gamma 3 region is an inducible promoter: synergistic activation by CD40 ligand and IL-4 via cooperative NF-kappa B and STAT-6 binding sites. J Immunol 1999;162:5327–36.
[50] Shankaranarayanan P, Chaitidis P, Kuhn H, Nigam S. Acetylation by histone acetyltransferase CREB-binding protein/p300 of STAT6 is required for transcriptional activation of the 15-lipoxygenase-1 gene. J Biol Chem 2001;276:42753–60.
[51] Delphin S, Stavnezer J. Characterization of an interleukin 4 (IL-4) responsive region in the immunoglobulin heavy chain germline epsilon promoter: regulation by NF-IL-4 a C/EBP family member andNF-kappaB/p50. J Exp Med 1995;181:181–92.
[52] Stavnezer J. Antibody class switching. Adv Immunol 1996;61: 79–146.
[53] Mikita T, Kurama M, Schindler U. Synergistic activation of the germline epsilon promoter mediated by Stat6 and C/EBP beta. J Immunol 1998;161:1822–8.
[54] Seidel HM, Milocco LH, Lamb P, Darnell Jr JE, Stein RB, Rosen J. Spacing of palindromic half sites as a determinant of selective STAT DNA binding and transcriptional activity. Proc Natl Acad Sci 1995;92:3041–5.
[55] Mikita T, Campbell D, Wu P, Williamson K, Schindler U. Requirements for interleukin-4-induced gene expression and functional characterization of Stat6. Mol Cell Biol 1996;16:5811–20.
[56] Jenkins K, Javadi M, Borghaei RC. Interleukin-4 suppresses IL-1-induced expression of matrix metalloproteinase-3 in human gingival fibroblasts. J Periodontol 2004;75:283–2891.
[57] Ameixa C, Friedland JS. Down-regulation of interleukin-8 secretion from mycobacterium tuberculosis-infected monocytes by interleukin-4 and -10 but not by interleukin-13. Infect Immunol 2001;69:2470–6.
[58] Crabtree GR, Olson EN. NFAT signaling: choreographing the social lives of cells. Cell 2002;109(Suppl):S67–79.
[59] Gingras S, Cote S, Simard J. Multiple signal transduction pathways mediate interleukin-4-induced 3beta-hydroxysteroid dehydrogenase/Delta5-Delta4 isomerase in normal and tumoral target tissues. J Steroid Biochem Mol Biol 2001;76:213–25.
[60] Loyer P, Ilyin G, Abdel Razzak Z, Banchereau J, Dezier JF, Campion JP, et al. Interleukin 4 inhibits the production of some acute-phase proteins by human hepatocytes in primary culture. FEBS Lett 1993;336:215–20.