Regulation of Spi 2.1 and 2.2 gene expression after turpentine inflammation: discordant responses to IL-6

SUSAN A. BERRY,1,2 PEARL L. BERGAD,1 ALLISON M. STOLZ,1 HOWARD C. TOWLE,2,3 AND SARAH JANE SCHWARZENBERG1,2

Departments of 1Pediatrics and 3Biochemistry and 2Institute of Human Genetics, University of Minnesota, Minneapolis, Minnesota 55455

Berry, Susan A., Pearl L. Bergad, Allison M. Stolz, Howard C. Towle, and Sarah Jane Schwarzenberg. Regulation of Spi 2.1 and 2.2 gene expression after turpentine inflammation: discordant responses to IL-6. Am. J. Physiol. 276 (Cell Physiol. 45): C1374–C1382, 1999.—The rat serine protease inhibitor (Spi) genes Spi 2.1 and 2.2. The nucleic acid and deduced amino acid sequences of these genes suggest they are members of the serpin gene family. The serpin gene family encodes a large number of proteins that share significant sequence homology but perform diverse biological roles. We have characterized the physiological regulation of rat Spi 2.1 and 2.2 mRNAs. Because Spi 2.1 is dependent on growth hormone (GH) for maximal hepatic mRNA content (24, 40), it has been used to study hepatic gene regulation by GH. In contrast, Spi 2.2 mRNA is not GH dependent (32).

Despite significant promoter sequence similarity, hepatic Spi 2.1 and 2.2 mRNAs have dramatic divergence in their responses to an inflammatory stimulus. Hepatic Spi 2.2 mRNA increases 10-fold within 12 h of inflammation induced by subcutaneous turpentine injection, whereas Spi 2.1 mRNA decreases to levels ∼25% of control values 48 h after stimulus (33). Spi 2.2 is thus a positive hepatic acute phase reactant, whereas Spi 2.1 is one of a small number of hepatic proteins that are negative acute phase reactants (33). Intracellular signaling by both GH and inflammatory mediators involves the actions of latent cytoplasmic signal transducer and activation of transcription (STAT) proteins. STAT proteins are tyrosine phosphorylated in response to a number of cytokine-receptor interactions. STAT3 was isolated from mouse livers following treatment with interleukin (IL)-6 and was initially called APRF, for acute phase response factor (1). STAT5, originally identified as a prolactin-induced factor, is activated in response to GH and binds to the Spi 2.1 promoter to mediate its transcriptional induction. However, GH is known to cause tyrosine phosphorylation of multiple STAT proteins, including STAT3 (5, 8, 15). Because Spi 2.1 and Spi 2.2 have divergent responses to inflammatory stimulation, we were interested in examination of potential STAT binding to the Spi 2.1 and 2.2 promoters after turpentine treatment to examine the specificity of differential gene expression in the Spi 2 gene family. As the proximal promoters of these genes differ only at putative STAT binding sites, we hypothesized that the hepatic APR following turpentine injection would activate STAT proteins and that differences in binding of STAT proteins would be critical to specificity and differential regulation of Spi 2 gene expression. To facilitate the examination of these model genes using a manipulatable in vitro system, we used hormone- and cytokine-treated primary hepatocytes to complement our previous studies in whole animals with the initial
hypothesis that IL-6 would be the primary cytokine regulating the APR of the Spi 2 genes.

METHODS

Induction of the hepatic APR by turpentine. Adult male rats (either hypophysectomized or normal) were sedated, injected subcutaneously with 0.5 ml turpentine/100 g body wt, and killed at intervals after injection. Use of hypophysectomized animals permitted examination of the STAT response without the effects of pituitary hormones except as supplied exogenously. For animals treated with GH, 150 µg human recombinant GH/100 g body wt were administered intraperitoneally 1 h before death. The livers were removed, and crude nuclear extracts were prepared as described (7). All animal studies were evaluated and approved by the Institutional Animal Care and Use Committee of the University of Minnesota.

Electrophoretic mobility shift assays. Probes used in electrophoretic mobility shift assays (EMSA) included a high-affinity sis-inducible element (SIEa; 5’-GTGACAGCTTCCCCGTAAATCGTCA-3’) (14) that binds STAT1 and STAT3 (5) and a fragment of the Spi 2.2 gene spanning −137 to −109 (5’-GAGTCCATTTCCCAAGATCATCCTGGTC-3’). This fragment is derived from the Spi 2.2 promoter region corresponding (27) to the Spi 2.1 GH response element (GHRE) (39) and contains a single γ-activated sequence (GAS; underlined in the element sequence), a feature noted in genes activated by STAT proteins. An oligonucleotide encompassing the Spi 2.1 GHRE was used for EMSA to monitor for STAT5 binding.

EMSA was performed as described (7) in a buffer containing 20 mM HEPES (pH 7.6), 1 mM MgCl2, 0.1 mM EDTA, 4% Ficoll 400, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride. For assays including antibodies, a STAT3 antibody (AbN) was the kind gift of Dr. David Levy (Department of Pathology and Kaplan Comprehensive Cancer Center, New York University School of Medicine, New York, NY) (29). This antibody recognizes both STAT1 and STAT3. The STAT1 antibody was obtained from Transduction Laboratories (Lexington, KY) and the STAT5b antibody from Santa Cruz Biotechnology (Santa Cruz, CA). All EMSA experiments were repeated a minimum of three times using extracts from separate individual animals, with representative experiments shown.

For comparison with extracts after turpentine stimulation of normal or hypophysectomized male rats, or IL-6 treatment of hepatocytes, GH injections were also given to either normal or hypophysectomized male rats. Because of differences in the responses of STAT proteins dependent on the pulsatile secretion of GH in intact male rats and a relative desensitization of STAT1 and STAT3 activation in normal rats (28), STAT5 levels are variable in normal male rats without GH treatment, and STAT1 and STAT3 are not seen in normal male rats treated with GH. STAT5 is reliably induced by GH treatment of either normal or hypophysectomized male rats; STAT1 and STAT3 are induced only after GH treatment of a hypophysectomized male rat. These extracts are included in assays shown as controls for STAT1, STAT3, and STAT5 migration on EMSA.

Plasmid construction and hepatocyte assays. The construction of Spi 2.2 (−319 to +85) into the Pst I sites of the parent chloramphenicol acetyltransferase (CAT) plasmid pCAT(An) was as described previously for other constructs (5). Spi 2.1 constructs [p(−275 to +85)-Spi 2.1-CAT (Spi-A-CAT), del (−149 to −102)-Spi 2.1-CAT (Spi-O-CAT), and a construct containing a mutation of the 3’ GAS site of the GHRE (Spi-E-CAT)] have been described previously (5, 39). An additional construct mutating the 5’ GAS site (underlined) of the GHRE was also prepared, containing the mutated GHRE sequence 5’-AAGCGCTTCGAGTCCATGTTTCTGAGAAAT-TCTAGAGTCTGGCCA-3’ (Spi-G-CAT). This mutation completely abolishes the response to GH in transfection assays (Bergad, Towle, and Berry, unpublished observations). All sequences were confirmed by DNA sequencing. All experiments were repeated using independent plasmid preparations to ensure reproducibility of the response of each construct.

For transfection assays, hepatocyte mRNA quantitation, and hepatocyte nuclear extract preparation, primary hepatocytes were isolated from male Sprague-Dawley rats (~200 g) using a collagenase perfusion method and plated at a density of 3.5 × 105 cells/60-mm Primaria culture dish. Functional assays in primary rat hepatocytes were carried out as previously described (34). After attachment and transfection (if performed), cells were cultured in the presence or absence of GH (50 or 500 ng/ml) and/or IL-6 (1.25–20 ng/ml). In addition, 500 µg/ml Matrigel (Life Technologies, Grand Island, NY) was added to the medium (35). For transfection studies, at the end of 48 h, the cells were harvested for CAT assay, and these results were expressed as percentage conversion of chloramphenicol to its acetylated forms as determined by phosphor screen autoradiography (Molecular Dynamics, Sunnyvale, CA). Each experiment was repeated three times with freshly isolated hepatocytes.

For hepatocyte mRNA isolation, the cells were cultured for 24 h after addition of hormones. They were then harvested, and total RNA was extracted (9). Northern blots were prepared and hybridized first to a Spi 2.1-specific oligonucleotide (33) and then simultaneously to a cDNA specific for the 3’ untranslated region of Spi 2.2 mRNA (31) and a cDNA for glyceraldehyde-3-phosphate dehydrogenase (GAPDH; to control for gel sample loading) using conditions as described (6). Quantitation of Spi 2.1 and 2.2 mRNA was determined by phosphor screen autoradiography and normalized to GAPDH mRNA quantities.

For nuclear extract preparation from hepatocytes, the cells were harvested at intervals of 15, 30, 60, and 120 min after addition of hormones, and nuclear extracts were prepared (2).

RESULTS

Activation of hepatic STAT3 by turpentine stimulation. We speculated that the physiological response to turpentine stimulation would include rapid activation of STAT proteins, particularly STAT3. To examine the time course of STAT protein activation after turpentine stimulation, normal male rats were injected with subcutaneous turpentine and killed at intervals after treatment. Hepatic nuclear extracts were prepared and tested for the presence of STAT protein binding using EMSA assays. Binding assays were performed using SiEa, a high-affinity sis-inducible element known to bind to STAT1 and STAT3, as a probe (Fig. 1A.) EMSA of hepatic nuclear extracts from untreated animals did not display any protein-DNA complexes. However, by 2 h after turpentine treatment, a single complex that binds to SiEa was present. The intensity of binding of this complex was further increased at 4 h after treatment. EMSA using these extracts and labeled GHRE from the Spi 2.1 promoter (which binds STAT5 and not STAT1 or STAT3) showed no changes in binding from the low levels of basal STAT5 binding (data not shown).
To determine which STAT protein was binding to the SIEa in these assays, antibodies to STAT proteins were added to the EMSA reaction to determine whether an alteration in mobility of the complex in the gel would result. Figure 1B shows that neither addition of STAT1 antibody nor addition of STAT5 antibody to the binding reaction resulted in altered mobility of the DNA-protein complex, but addition of an antibody recognizing both STAT1 and STAT3 (AbN) resulted in retardation of the mobility of the entire complex. Thus the turpentine-stimulated complex capable of binding to the SIEa contains STAT3 and not STAT1 or STAT5.

Pituitary dependence of STAT binding activity in hepatic nuclear extracts. To evaluate whether there were pituitary-dependent differences in the STAT proteins activated after turpentine treatment, hepatic nuclear extracts from hypophysectomized and normal male animals treated with GH, with turpentine, or with both were used in EMSA with labeled SIEa and GHRE. Figure 2 shows that small amounts of activated STAT3 are present in hypophysectomized animals. Injection with GH results in activation of STAT1, STAT3, and STAT5 binding in hypophysectomized animals, as noted previously (5), but only STAT5 is activated in normal animals. Turpentine injection results in increased binding of STAT3 in both normal and hypophysectomized animals. STAT5 binding is not seen in hypophysectomized, turpentine-treated animals, and no increase over basal STAT5 binding is seen in normal animals treated with turpentine. The addition of GH treatment 4 h after turpentine treatment of a hypophysectomized animal results in a binding pattern that is not different from that seen after GH treatment alone. Thus there are no substantial differences in the pattern of turpentine-stimulated STAT protein binding attributable to GH state.

Potential for STAT binding to the Spi 2.2 GAS element. The activation of STAT3 after turpentine stimulation of inflammation suggests the potential for STAT3 to function as an activating protein for Spi 2.2 transcription. To determine whether STAT proteins can bind to the Spi 2.2 promoter, we did EMSA using a fragment of the Spi 2.2 promoter containing a GAS element. This element is found at the same promoter position as the GHRE in Spi 2.1 but contains only a single GAS site, rather than the paired GAS elements of the Spi 2.1 promoter. This was bound to hepatic nuclear extracts from hypophysectomized male rats treated with either GH or with turpentine. Four statespecific complexes bound to the Spi 2.2 GAS element are present in extracts from GH-treated hypophysectomized animals. These complexes migrated at positions expected for STAT5 dimers, STAT3 homodimers, STAT1-STAT3 heterodimers, and STAT1 homodimers (top to bottom positions in Fig. 3A). Addition of appropriate antibodies to various STAT proteins confirmed that the
GH-induced protein-DNA complexes include STAT1, STAT3, and STAT5 (Fig. 3, B and C). However, only one state-specific complex migrating at the position of STAT3 homodimers was present after turpentine stimulation. The Spi 2.2 promoter GAS site thus can bind three STAT proteins, although the potential for these varied binding patterns is dependent on the pituitary state of the animal. Because Spi 2.2 mRNA can be
induced in the absence of GH and because only binding of STAT3-containing complexes is found after turpentine treatment of hypophysectomized rats, STAT3 is likely to be the physiologically relevant binding factor for induction of the Spi 2.2 promoter.

Development of an in vitro APR model: IL-6 action in induction of Spi 2.2 and 2.1 mRNAs. Turpentine injection results in production of IL-6 and other cytokines (10, 11). We noted that STAT3 was specifically activated after turpentine treatment, presumably due to stimulation of IL-6 by the tissue injury caused by the turpentine injection. We inferred that, in turn, Spi 2.2 transcription could be induced by IL-6-activated STAT3 binding to the Spi 2.2 GAS element. To complement these in vivo observations and to develop a model system that would allow both investigation of the roles of individual cytokines in the hepatic APR and studies of the responses of individual promoter elements, we used primary hepatocytes. To more directly evaluate the role of IL-6 in induction of Spi 2.2 mRNA, we examined the endogenous induction of this mRNA in primary hepatocyte cultures treated with IL-6 because our data to this point suggested that IL-6 was the primary cytokine required for Spi 2.2 induction. To examine the specificity of the response of the Spi 2.2 promoter, especially as it contains a site that is capable of binding to a variety of STAT proteins, the effect of GH treatment on Spi 2.2 mRNA was also evaluated. As seen on a representative Northern blot (Fig. 4, top), Spi 2.2 mRNA was very low in RNA isolated from untreated hepatocytes but was clearly present in RNA from cells treated with IL-6, producing increases in Spi 2.2 mRNA (4.36 ± 0.18-fold change from untreated hepatocytes, mean ± SE, n = 10). As anticipated on the basis of previous studies, GH treatment did not result in a significant increase in Spi 2.2 mRNA quantities (1.10 ± 0.14-fold, n = 7), nor did it alter the effect of IL-6 when cells were treated with both GH and IL-6 (4.30 ± 0.06-fold, n = 4). GH effectiveness was confirmed by an observed increase in Spi 2.1 mRNA levels in these hepatocytes (4.60 ± 0.038-fold, n = 3). In contrast to the observed induction of Spi 2.1 mRNA by GH, IL-6 did not increase Spi 2.1 mRNA (0.63 ± 0.014-fold, n = 6).

In vitro action of IL-6 in activation of STAT3 binding. To determine more directly whether IL-6 is an activating ligand for STAT3 in cultured primary hepatocytes, we performed EMSA with nuclear extracts of hepatocytes treated with IL-6 or GH. Figure 5 shows the time course of STAT3 activation by IL-6. Within 15 min of IL-6 treatment, activated STAT3 was already present in the nuclear extracts. Between 30 min and 2 h there was also a transient appearance of STAT1 and STAT1-STAT3 heterodimers. Under these conditions, however, despite the activation of STAT1, STAT3, and STAT5 by GH in vivo and the responsiveness of hepatocytes to GH evidenced by an increase in Spi 2.1 mRNA, we did not observe activation of STAT3 by GH.

Action of IL-6 on the Spi 2.2 and 2.1 promoters. To determine whether the observed increase in Spi 2.2 mRNA is due primarily to transcriptional activation of the Spi 2.2 promoter, induction of a Spi 2.2-CAT construct was examined in hepatocytes treated similarly to those noted above. For this purpose a construct containing a Spi 2.2 promoter fragment (−319 to +85; includes the Spi 2.2 GAS element) coupled to CAT was introduced. A 28-fold induction of CAT protein was observed after IL-6 treatment (Fig. 6A). Again, as a control for specificity, GH failed to induce Spi 2.2-CAT and did not augment the effects of IL-6. Thus the observed increase in Spi 2.2 mRNA in the treated...
hepatocytes is due at least in part to transcriptional activation of its proximal promoter that can be stimulated by IL-6.

We anticipated that IL-6 would not activate Spi 2.1-CAT constructs because decreases in Spi 2.1 mRNA occur in animals treated with turpentine and Spi 2.1 mRNA did not increase in hepatocytes treated with IL-6. However, when a construct containing the promoter fragment of Spi 2.1 known to respond to GH (−275 to +85, Spi-A-CAT) was used in transfection assays with IL-6 treatment, a 5.4-fold induction of Spi 2.1-CAT was observed. Deletion of the portion of this plasmid containing the GHRE (del −149 to −102, Spi-O-CAT) ablated this response, as did selective mutation of either the 3′ (Spi-E-CAT) or 5′ (Spi-G-CAT) GAS elements in the paired GAS site of the Spi 2.1 GHRE (Fig. 6B). This spectrum of response is essentially identical to the one that we have reported previously after GH treatment. Thus the GHRE is not a GH-specific element but also responds actively to IL-6 under these cell culture conditions. In addition, in this cell culture model with these promoter fragments, IL-6 alone is insufficient to simulate the negative response to turpentine-induced inflammation found for Spi 2.1 in whole animals, although it is sufficient to simulate the positive response of Spi 2.2.

DISCUSSION

As a part of the systemic response to inflammation, the rate of synthesis of a selected group of hepatic proteins is altered. The hepatic APR is stimulated during any tissue injury, including burns, surgery, parturition, and sepsis (19, 22, 23). The spectrum of proteins altered by the APR is the same regardless of the specific injury eliciting the response, with the quantitative response resulting from the summation of the complex signals from various cytokines and hormones. The major mediators of change in hepatic gene response during inflammation include IL-6, IL-1β, tumor necrosis factor-α (TNFα), interferon-γ, and glucocorticoids, although other cytokines and hormones are also involved. Many of these cytokine mediators are derived from stimulated monocytes and macrophages. The network of cytokines released during inflammation have both overlapping and competing properties. The concentration of the cytokines and hormones, the sequence in which they reach the liver, and their interactions all affect the response of any individual protein in the APR (20).

Transcriptional regulation of the APR is dependent on DNA elements found in cis in the promoters of acute phase reactant genes. Studies in rat hepatoma cell lines identified two distinct groups of hepatic acute phase reactants: group 1 proteins are regulated by a combination of IL-1, IL-6, and glucocorticoids, and group 2 proteins are regulated by IL-6, with or without glucocorticoids, but without a requirement for IL-1 (4, 12). Correspondingly, two DNA response elements were identified: group 1 genes contain an element that binds a member of the CCAAT/enhancer-binding protein (C/EBP) family, NF-IL-6 (C/EBPβ). In response to IL-6-mediated glycoprotein 130 homodimerization, the
ras-mitogen-activated protein kinase system is activated, eventually activating NF-IL-6 (18). Group 2 genes contain an element that binds STAT3 (17). This DNA element (type 2 IL-6 response element) is similar to other GAS elements known to bind members of the STAT family (41).

Injection of turpentine is a reproducible method for eliciting the APR in vivo (26, 33). In this model, hepatic mRNA levels of typical APR products are maximally changed by 24 h (26, 33, 30). Injection of IL-6 into rats results in a more rapid increase in IL-6-responding APR gene products than turpentine injection; this difference is likely due to the requirement for intermediate stimulation of IL-6 by the tissue injury associated with turpentine injection (37). Induction of the APR using turpentine in this study resulted in the rapid appearance of activated STAT3 as evidenced by binding to both the SIEa, a GAS element that recognizes only STAT1 and STAT3, and to the Spi 2.2 GAS site. The relatively rapid appearance of activated STAT3 after turpentine stimulation, and its ability to bind to the GAS site in the Spi 2.2 promoter, could result in activation of Spi 2.2 transcription and account, at least in part, for the increases observed in mRNA levels at 12 h after injection. This pathway has been recapitulated in primary hepatocyte cultures in which the rapid appearance of STAT3 and the response of Spi 2.2 mRNA and Spi 2.2-CAT promoter constructs to IL-6 stimulation have been documented. These observations further support the conclusion that STAT3 activation in response to turpentine-stimulated IL-6 is the likely mechanism for increases in Spi 2.2 mRNA after turpentine stimulation.

The Spi 2.2 GAS element is capable of binding STAT1 and STAT5 in addition to STAT3 and thus can bind all three STAT proteins activated by GH (5, 8, 25). Despite this, we do not find evidence that endogenous STAT activation from GH treatment results in stimulation of Spi 2.2 transcription or that either STAT1 or STAT5 is necessary for Spi 2.2 mRNA induction. One source of this differential response to GH could be the architecture of the GAS elements in the Spi 2.1 and 2.2 promoters. These promoters have nearly identical (2-nucleotide difference) GAS elements. The Spi 2.1 promoter also has a second 5'-GAS element in addition to the primary GAS element conserved in both Spi 2.1 and Spi 2.2 promoters. These promoters have nearly identical (2-nucleotide difference) GAS elements. The Spi 2.1 promoter requires the presence of these paired GAS elements in its promoter, but the second, 5'-element is disrupted in the Spi 2.2 promoter. Furthermore, we have shown previously that the 3' GAS element in Spi 2.1 as an isolated GAS site binds STAT5 only weakly (5); it also fails to bind STAT1 or STAT3. The two-nucleotide difference in the otherwise conserved 3' GAS site of Spi 2.1 (Spi-E-CAT), and a mutation of 5' GAS site (Spi-G-CAT).

The profile of STAT proteins critical to Spi 2.2 expression in the APR is succinct despite the capacity of its GAS element to bind more than one STAT protein. In experiments using overexpression of STAT proteins, Kordula et al. (21) observed activation of the Spi 2.2 promoter by STAT5b. However, under GH conditions that result in substantial induction of Spi 2.1 promoter-CAT constructs (5), the levels of STAT proteins induced by GH do not induce a Spi 2.2 promoter-CAT construct. Furthermore, hepatic nuclear extracts from hypophysectomized animals treated with turpentine do not contain factors that bind to elements that recognize STAT5 specifically, despite the observation that this treatment increases expression of Spi 2.2 mRNA (33). There is also no increase in STAT5 binding in extracts from normal animals treated with turpentine. Thus STAT3 is an essential element in the positive APR to IL-6, as exemplified by the Spi 2.2 mRNA response. The partic-
pation of other endogenous STAT proteins in the Spi 2.2 response is less likely.

One goal of these studies was generation of an in vitro model of APR induction using primary hepatocytes. Such a model system would be complementary to whole animal studies because it would allow manipulation of the hormonal milieu contributing to the APR and specifically allow study of cis elements of gene promoters important in the APR. An additional side benefit is that it would reduce the number of invasive treatments that would be required to do such studies in whole animals. Although an IL-6 increase after turpentine injection can account for the positive APR of Spi 2.2, giving IL-6 as an isolated cytokine in the hepatocyte model does not reproduce the negative APR observed after turpentine injection in the whole animal. If IL-6 were the sole effector of the negative APR observed for Spi 2.1 in vivo, we should have seen repression by IL-6 of GH induction of Spi 2.1-CAT constructs in vitro. Instead, an increase in gene expression driven by a fragment of the Spi 2.1 promoter was observed. The paradoxical IL-6 induction of the Spi 2.1 promoter fragment in hepatocyte culture is mediated via the paired GAS sites of the Spi 2.1 GHRE. Wood et al. (38) showed that neither STAT1 nor STAT3 induces transcription via the Spi 2.1 promoter and suggested that this promoter is STAT5 specific. This suggests that IL-6 may activate STAT5 and in turn activate this Spi 2.1 promoter construct via the paired GAS element. Because an induction rather than a decrease was observed, there could be elements present in the native Spi 2.1 gene that modify the responses we observed using a promoter fragment encompassing only sequences from −275 to +85. However, similar results were seen using a fragment −3200 to +85 (not shown). If IL-6-regulated inhibitory sequences are important in the negative APR of Spi 2.1, they are not present in these portions of the gene.

The observed induction of the reporter gene expression driven by the Spi 2.1 promoter fragment is discordant with the decrease in Spi 2.1 mRNA expression observed after turpentine treatment and with a lack of increase of Spi 2.1 mRNA expression after IL-6 treatment of cultured hepatocytes. The profile of cytokines induced by turpentine treatment, although it includes IL-6, therefore also likely includes a factor that suppresses Spi 2.1 expression. Downregulation of the APR may be regulated in part by TNFα and IL-1β (13). We propose that the regulation of the observed decrease of Spi 2.1 mRNA in the APR is thus likely to be due to the interaction of more than one cytokine and hormonal stimulus and, unlike the upregulation of Spi 2.2 mRNA, is not dependent on induction of IL-6 alone.

Despite the demonstrable physiological regulation of the Spi 2.1 gene by GH, its promoter can be activated by other ligands after transfection into cultured cells. Wood et al. (38) observed a similar induction of this promoter in COS-7 cells cotransfected with STAT5 expression plasmid and stimulated with erythropoietin. In cultured cells, then, other ligand-receptor activation that results in STAT5 phosphorylation can induce expression from a Spi 2.1 promoter construct containing the paired GAS sites that mediate GH response. A similar observation was noted for activation of the β-casein promoter by IL-6 in mammary epithelial cells (36). Thus any studies to evaluate the effects of specific hormones in vitro need to be carefully correlated with appropriate in vivo studies if physiological relevance is at issue. The specific effects of IL-6 as an isolated cytokine on Spi 2.1 gene expression in vivo are not known.

The mechanisms that result in differential STAT activation and, in turn, differential expression of the divergently regulated Spi 2 genes after an acute phase stimulus are complex. Quantitative or qualitative differences in the various STAT proteins available for binding may result in differential activation of Spi 2 gene promoters, and differences in DNA element architecture or the presence of other nuclear factors may modulate the responses of Spi 2 promoters after induction of the APR. Further work examining these possibilities in this provocative model system should be of value in defining these differences in regulation. It will be necessary, however, to generate a hepatocyte model that more faithfully reproduces the in vivo response to turpentine or other acute phase stimuli to establish the mechanism of the negative APR of Spi 2.1, as treatment with IL-6 alone will not faithfully reproduce the negative APR.

We thank Elizabeth Kaytor for preparation of primary hepatocytes.

This work was performed with the support of National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-32817 and the Viking Children’s Fund.

Address for reprint requests and other correspondence: S. J. Schwarzenberg, Dept. of Pediatrics, University of Minnesota, 420 Delaware St. SE, Box 185 FUMC, Minneapolis, MN 55455 (E-mail: schwa005@tc.umn.edu).

Received 27 August 1998; accepted in final form 24 February 1999.

REFERENCES


