Accelerated Publications

Analysis of Complement Factor H mRNA Expression: Dexamethasone and IFN-γ Increase the Level of H in L Cells†,‡

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ABSTRACT: Murine complement protein H is encoded by a 100-kb gene on chromosome 1. A 3.2-kb fragment of the 5′ flanking region of the H gene was sequenced, and two transcription start sites for this gene were identified by RNase protection and S1 nuclease analyses, each of which had upstream TATA and CAAT boxes. This region shares sequence homology with known regulatory elements, including the SV40 enhancer consensus, the Sp1 binding site, and two glucocorticoid-responsive core elements (GRE). Tissue and cell-line specificity has been examined by Northern analysis, and the 4.4-kb full-length H messenger RNA was identified in liver, kidney, spleen, thymus, liver cell line 1469, and L cells. IFN-γ did not induce H mRNA expression in the macrophage cell line P388D.1 but had a positive effect on both the mRNA and protein levels of H in L cells. PMA, LPS, and vitamin D did not increase H mRNA levels in L cells. Pursuant to the discovery of two GRE in the 5′ regulatory region of the H gene, we examined the effects of glucocorticoids on H mRNA expression. Dexamethasone (10^{-7} M) was found to increase markedly the levels of H mRNA and protein after 24 h of incubation, and the effect on the mRNA was detectable by 30 min. The fact that H is a down-regulator of complement activation is consistent with the known immunosuppressive role of glucocorticoids. To our knowledge, this is the first time that dexamethasone has been shown to increase the levels of a complement protein. Presently, we are using the luciferase system to delineate the sequences important for the regulation of the factor H gene.

Complement component factor H (H) is a plasma protein involved in the regulation of the alternative pathway of complement activation. It has two mechanisms through which it acts to control activation. The first, termed decay acceleration, involves its ability to bind to C3b,Bb or C3b,Bb,P, the C3 convertases of the alternative pathway of complement activation, and displace the Bb bound to C3b, thus destroying the convertase (Whaley & Ruddy, 1976; Weiler et al., 1976). The other functional activity of H in the control of complement activation is to serve as a cofactor in the complement factor I mediated cleavage of C3b (Pangburn et al., 1977). This cleaved C3b (iC3b) is no longer able to form a C3 convertase.

The major site of synthesis for many complement components, including H, is the liver (Morris et al., 1982; Schwaeble et al., 1987). Synthesis of H in humans has also been reported in the promonocyte cell line U937 (Malhotra & Sim, 1985), skin fibroblasts (Katz & Strunk, 1988) and endothelial cells (Ripoche et al., 1988b; Brooimans et al., 1989). Conflicting reports have been made as to whether peripheral blood monocytes synthesize H; one group (Whaley, 1980) detected H synthesis whereas another (Katz & Strunk, 1988) did not. Various agents have been used to modulate H expression, including IFN-γ, IL-1, LPS, TNF, and T-cell growth factor (TCGF) (Katz & Strunk, 1988; Brooimans et al., 1989). H expression in human cells was shown to increase with both IFN-γ and TCGF, whereas TNF, LPS, and IL-1 had little effect. The isolation of a murine cDNA clone specific for H (Kristensen & Tack, 1986) has allowed us to examine the tissue-specific mRNA expression in mice and to determine the effect on H mRNA levels of various stimulators of the immune response. In addition, we have recently isolated 17 overlapping clones containing the murine H genomic sequence (Vik et al., 1988) and determined the structure of this gene. This has enabled us to examine the sequence of the 5′ regulatory region of the H gene and to compare it to other 5′ flanking genomic sequences.

Experimental Procedures

Cell Culture. All cell lines were cultured in DMEM containing 10% fetal bovine serum except for J774A.1 cells, which were cultured in a mixture of DMEM/RPMI (1:1). Cells were grown to confluency, the various modulating agents were then added, and the cells were incubated for up to 48 h.

Isolation and Analysis of Total and Poly(A)+ RNA. Total RNA was isolated from various cell lines and tissues by the guanidinium thiocyanate/cesium chloride method (Chirgwin et al., 1979). Poly(A)+ RNA was selected by using an oligo(dT) column (Type III; Collaborative Research, Waltham, MA) as described (Maniatis et al., 1982). Northern analysis was performed as described (Gehring et al., 1987), and slot blot analysis was performed with a Hybri-Slot Manifold slot blotter apparatus (BRL, Gaithersberg, MD) as described by the manufacturer. DNA probes were radiolabeled by the random primer method (Feinberg & Vogelstein, 1983). The relative amount of radioactivity specifically bound to the filters was determined by analyzing the autoradiograph with an LKB UltroScan XL laser scanning densitometer and was normalized to the level of β-actin mRNA. The murine H cDNA probe

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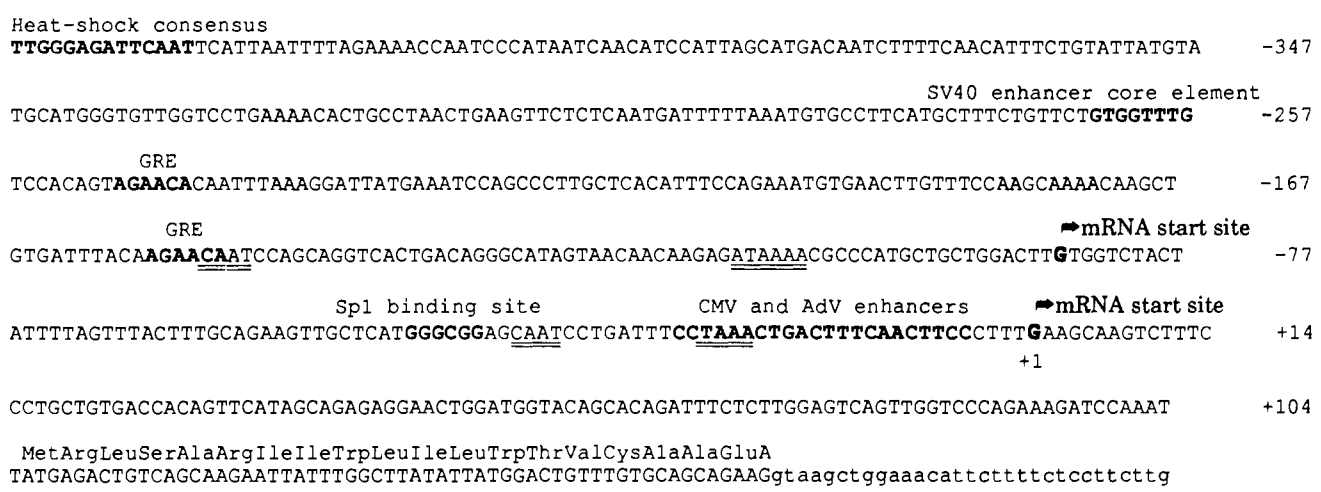

FIGURE 1: Sequence of the 5′ region of the H gene. The locations of the mRNA CAP sites as determined by RNase protection and S1 analyses are indicated by an outlined G. The amino acid sequence of the signal peptide is shown above the DNA sequence. Small letters indicate the beginning of the first intron. The putative TATA and CAAT boxes are double underlined and the cis-acting consensus sequences are in bold letters.

was a *Bam*HI fragment corresponding to positions 1041–2488 of the full-length sequence (Kristensen & Tack, 1986). Chicken β-actin cDNA was a gift from Dr. R. Ulevitch, and class II probe was a gift from Dr. A. Celada.

Western Blotting. Culture supernatants of L cells that had been incubated for 24 h in medium alone or in medium supplemented with 10^{-7} M dexamethasone or 500 units/mL IFN-γ were subjected to SDS/polyacrylamide gel electrophoresis and transferred to nitrocellulose as described (Burnette, 1981). The blots were developed with monospecific rabbit anti-murine H, biotin-goat anti-rabbit IgG (Boehringer Mannheim Biochemicals, Indianapolis, IN), avidin-alkaline phosphatase (Bio-Rad, Richmond, CA), and a substrate kit from Bio-Rad.

Nucleotide Sequencing. The 3.5-kb *Sal*I/*Xho*I fragment of the A-18 cosmid clone was "shotgun" cloned into M13mp8 and sequenced by a modification of the dideoxy chain termination procedure (Sanger et al., 1977) using a commercial sequenase kit (United States Biochemical, Cleveland, OH).

RNase Protection and S1 Nuclease Mapping. For the RNase protection analysis of H mRNA, the 3.5-kb *Sal*I/*Xho*I fragment containing the leader peptide was subcloned into the vector pBS/M13 (Stratagene, San Diego, CA). The plasmid was linearized at a convenient *Dra*II site and ^{32}P-labeled RNA complementary to H mRNA 854 nucleotides in length was generated from the T3 promoter. An aliquot of the T3-generated RNA was added to 10 μg of total RNA in a buffer containing 80% formamide, 40 mM PIPES, pH 6.7, 0.4 M NaCl, and 1 mM EDTA. The samples were heated at 85 °C for 5 min and then incubated at 45 °C overnight. They were diluted in a buffer containing 10 mM Tris-HCl, pH 7.5, 5 mM EDTA, and 300 mM NaCl, and RNase A (BMB) was added at 40 μg/mL for 1 h at 22 °C. The reaction was stopped by the addition of SDS to 0.5% and proteinase K to 166 μg/mL followed by incubation at 37 °C for 15 min. After phenol/chloroform extraction and ethanol precipitation the products of this reaction were electrophoretically separated on a 6% polyacrylamide/8 M urea sequencing gel. Dried gels were exposed to X-ray film with an intensifying screen at −70 °C.

For the S1 analysis of H mRNA, an M13 subclone from this region was selected whose primer-extended product was complementary to the mRNA of interest. A radiolabeled copy was made with [^{32}P]dCTP by primer extension starting at the universal priming site of the M13 subclone. This mixture was digested with *Eco*RI, resulting in a strand of 402 nucleotides in length. This radiolabeled strand was separated from the M13 phage DNA by electrophoresis through an alkaline agarose gel, and 2×10^4 cpm was added to 5 μg of poly(A)+ RNA and hybridized in 30 μL of 80% formamide, 0.4 M sodium chloride, 1 mM EDTA, and 40 mM PIPES, pH 6.4. The mixture was incubated at 75 °C for 15 min and then at 50 °C overnight. The sample was added to 350 μL of 0.25 M sodium chloride, 2 mM zinc acetate, 30 mM sodium acetate, pH 4.5, and 5 μg of sonicated salmon sperm DNA containing from 1000 to 2000 units of S1 nuclease (BMB) and incubated at 37 °C for 1 h. The mixture was then subjected to phenol/chloroform extraction followed by ethanol precipitation. After being incubated at 100 °C for 2 min, the samples were separated on a 5% acrylamide/7 M urea sequencing gel for 3 h at 50 W. The M13 subclone was also sequenced by the dideoxy chain termination method and run on the same gel as a size standard.

Results

Sequence of the 5′ Terminal Region of the H Gene. The murine genomic cosmid clone A-18 had previously been shown to contain the sequence coding for the leader peptide of H (Vik et al., 1988). A 3.5-kb *Sal*I/*Xho*I fragment from the 5′ end of this clone, which hybridized to a 20-mer oligonucleotide corresponding to the leader peptide, was isolated, and its nucleotide sequence was determined. Several regions corresponding to the cDNA sequence were identified, including the 5′ untranslated region, the leader sequence, and 4 bp of coding sequence for the mature protein (Figure 1). The translation start site was located 267 bp upstream of the *Xho*I site.

Mapping the Transcription Start Site(s) of the H Gene. To determine the transcription start site for this gene, a combination of RNase protection assay and S1 nuclease mapping was used (Figure 2). In the RNase protection assay, a major band of 165 nucleotides was detected by autoradiography in both the liver and L-cell lanes and a second weaker band (possibly a doublet) of ~250 nucleotides was observed in the liver lane. To confirm these results, S1 nuclease analysis was performed. A major band of 101 nucleotides was detected by autoradiography in the liver and L-cell lanes, and a second weaker band (that could be a doublet) of 187 nucleotides was observed in the liver lane. The bands observed in each of these experiments identify the same two transcription start sites, which correspond to positions 105 and 195 nucleotides upstream of the first ATG (Figure 1). The major band corre-

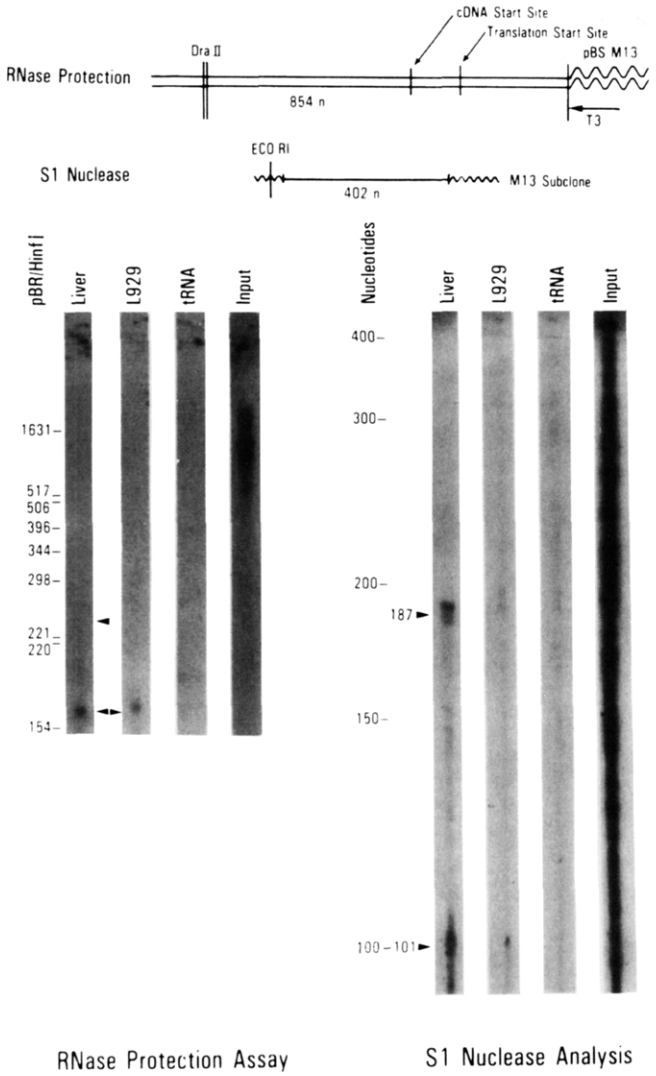

FIGURE 2: Mapping of the H transcription start site by RNase protection assay and S1 nuclease analysis. RNase protection assay. An 854-nucleotide ^{32}P-labeled RNA complementary to H mRNA was added to 10 μg of total RNA from murine liver (lane 1), L929 cells (lane 2), or tRNA (lane 3) and subjected to RNase A digestion as described under Experimental Procedures. The input probe of 854 nucleotides is shown in lane 4. The arrowheads depict the protected fragments. S1 nuclease analysis. Poly(A)+ RNA (5 μg) from murine liver (lane 1), L929 cells (lane 2), or tRNA (lane 3) was annealed to a radiolabeled fragment of DNA from this region and subjected to S1 nuclease digestion as described under Experimental Procedures. A dideoxy sequencing reaction was carried out on the same DNA fragment and run as a size marker. The right lane corresponds to the input probe of 402 nucleotides. The arrowheads depict the protected fragments.

sponding to position 105 has been assigned as nucleotide +1. Preliminary experiments indicate that this region also has promoter activity in the luciferase reporter gene system (data not shown).

Identification of Potential Cis-Acting Regulatory Elements. A search of the DNA sequence from the 5′ region of the murine H gene revealed the two common DNA sequence elements that are known to be important for proper transcription of many eukaryotic genes, the TATA and the CAAT elements (Efstratiadis et al., 1980; Maniatis et al., 1987). No consensus TATA element was found, but with respect to the major mRNA start site, at position −24 there is a TATA-box-like element, TAAA, and a CAAT box and GC-rich region are located at positions −38 and −46, respectively. The minor start site has an ATAAAA element and a CAAT box located 25 and 65 nucleotides upstream of it, respectively

Table I: Homologies between Sequences in the 5′ Flanking Region of the H Gene and Known Regulatory Sequences[a]

Heat-shock consensus	C T N G A A T N T T C N A G * * * * * * * * * * * * * * *
	-424 A T T G A A T C T C C C A A -437
Mouse cytomegalovirus enhancer 18 bp consensus	T C A A T A G G G A C T T T C C A B * * * * * * * * * * * * * * * * * *
	-26 C C T A A A C T G A C T T T C A A C -9
Adenovirus enhancer consensus	G G A A G - T G A A A * * * * * * * * * * *
	-5 G G A A G T T G A A A -15
SV40 enhancer core consensus	G T G G B B B G * * * * * * * *
	-264 G T G G T T T G -257
Sp1 binding site	G G G C G G * * * * * *
	-46 G G G C G G -41
Glucocorticoid responsive element (core)	A G R A C A * * * * * *
	-248 A G A A C A -243 * * * * * *
	-156 A G A A C A -151

[a] N, any base; B, A or T; R, A or G; −, gap introduced to maximize homology; *, designates homology.

(Figure 1). Which, if any, of these TATA- and CAAT-type elements is functional has not yet been determined.

The 3.5 kb of sequence from the H gene 5′ flanking region, including the first exon and part of the first intron, were examined for sequences similar to known enhancers. A number of potential enhancer sequence elements were identified and are listed by sequence and position in Table I. This list includes sequences that share identity with the SV40 enhancer (Gruss, 1984), the SV40 Sp1 protein binding site (Dynan & Tjian, 1985), the adenovirus enhancer (Hearing & Shenk, 1983), one of the motifs of the mouse cytomegalovirus enhancer (Dorsch-Häsler et al., 1985), the consensus sequence found in promoters of genes coding for the heat-shock proteins in a variety of organisms (Pelham, 1982), and the hexanucleotide TGTYCT, which is the glucocorticoid-responsive element (GRE) core homology (Karin et al., 1984; Payvar et al., 1983; Moore et al., 1985; Schütz, 1988).

H Expression in Murine Tissues and Cell Lines. Poly(A)+ RNA was isolated from murine liver, kidney, spleen, and thymus, subjected to RNA blot analysis, and probed with a radiolabeled H cDNA fragment. Four transcripts from liver RNA of 4.4, 3.5, 2.8, and 1.8 kb, respectively, hybridized to this probe. The 4.4-kb one, which represents the full-length H message, was also present in the other three tissues, and the 1.8-kb transcript was weakly detected in kidney (Figure 3). When total RNA from these tissues was used instead of poly(A)+, the 4.4-kb transcript was not observed in spleen or thymus nor was the 1.8-kb transcript observed in kidney (data not shown).

Six different cell lines were examined for their capacity to express H mRNA. Poly(A)+ RNA from these cell lines was isolated from equal amounts of total RNA and subjected to RNA blot analysis (Figure 4). Only two out of the six cell lines examined hybridized with the H cDNA probe. The fibroblast cell line L929 and the liver cell line 1469 expressed the 4.4-kb transcript, and in a longer exposure of the autoradiograph, the 1.8-kb transcript was also weakly detected in both cell lines (Figure 4A,B).

The liver cell line BNL.CL2 (Figure 4B), the B cell line A.20, and the macrophage cell lines P388D.1 (Figure 4C) and J774A.1 (Figure 4D) were negative for H mRNA expression. An attempt was made to stimulate the expression of H in these macrophage cell lines. P388D.1 macrophages were incubated

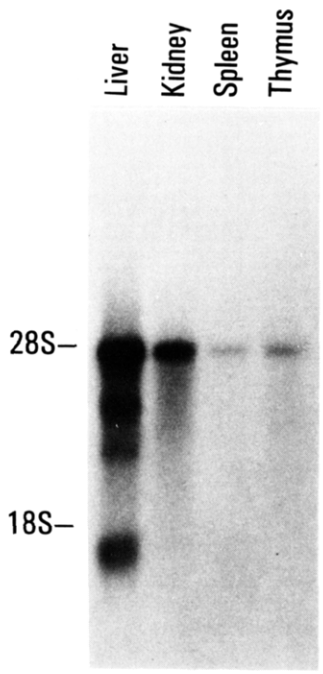

FIGURE 3: Northern blot analysis of poly(A)+ RNA from murine tissues. Poly(A)+ RNA (5 μg) from the indicated tissues was fractionated in a 1% agarose/formaldehyde gel, transferred to a nylon membrane, and hybridized with an H cDNA probe.

in medium alone or in medium containing IFN-γ (300 units/mL) for 24 h. Poly(A)+ RNA was isolated from 200 μg of total RNA and analyzed on a Northern blot, which also included a lane of poly(A)+ RNA from 200 μg of total RNA of the B cell line A.20. The H cDNA probe did not hybridize with the RNA in any of the three lanes (Figure 4C). The filter was reprobed with a class II major histocompatibility complex I-A_β cDNA, whose gene is known to be regulated by IFN-γ. I-A_β mRNA increases severalfold with respect to the control in P388D.1 macrophages that had been treated with IFN-γ, and the B cell line A.20 expressed constitutively high levels of I-A_β RNA. The macrophage cell line J774A.1 was stimulated with LPS (10 ng/mL), PMA (25 ng/mL), or vitamin D (10 ng/mL), but none of these modulators were able to induce H mRNA expression (Figure 4D).

L cells were examined for their capacity to be modulated for H mRNA expression by various external stimuli. L cells were incubated for 24 h in medium alone or in medium containing either IFN-γ (500 units/mL), PMA (100 ng/mL), LPS at three different concentrations (10, 50, and 100 ng/mL), vitamin D (10 ng/mL), or dexamethasone (10^{-7} M), and total RNA was examined by Northern blot analysis (Figure 5A). The results indicate that IFN-γ caused a 3–5-fold increase in H mRNA synthesis. However, PMA, LPS, and vitamin D, alone or in combination with IFN-γ, do not appear to change H mRNA levels in L cells. The levels of H mRNA in L cells treated with IFN-γ + LPS are comparable to those obtained in cells treated with IFN-γ only. L cells treated with dexamethasone for 24 h increased their H mRNA levels dramatically, and when IFN-γ was used in combination with dexamethasone to stimulate these cells, there was an apparent enhanced effect (Figure 5A). The amount of RNA in each lane was standardized by reprobing the Northern blots with chicken β-actin cDNA. After the autoradiographs were scanned, the increase in H mRNA levels was estimated to be more than 30-fold, compared to the control cells, when 10^{-7} M dexamethasone was used for 24 h. The dexamethasone and IFN-γ effects on L cells were further examined at the protein level. Western blot analysis was performed on the supernatant of cells that had been incubated in the absence or presence of dexamethasone or IFN-γ, using a monospecific rabbit anti-mouse H antisera. Both modulators elevated the production of H protein as detected by the increased intensity of the 150-kDa band (Figure 5B).

IFN-γ Concentration Dependence for the Increase of H

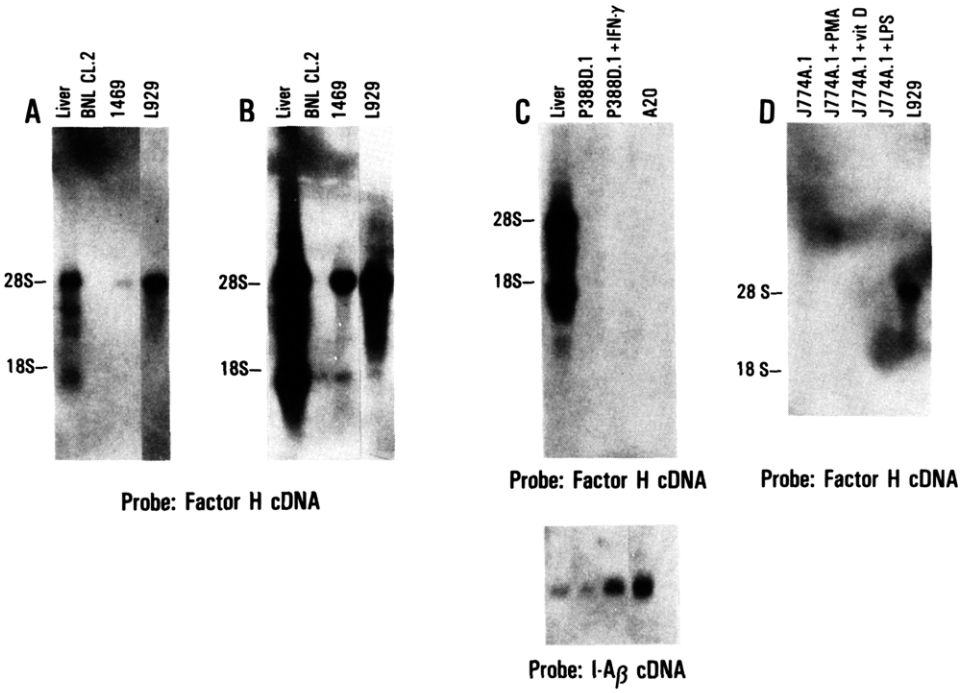

FIGURE 4: Northern blot analysis of poly(A)+ RNA from murine cell lines. (A) Poly(A)+ RNA (5 μg) from the indicated cell lines was analyzed as described in Figure 3. (B) Longer exposure of panel A to demonstrate the 1.8-kb transcript in the L929 and 1469 cell lines. (C) Effect of IFN-γ on H mRNA expression in murine macrophages. Poly(A)+ RNA from equal amounts (200 μg) of total RNA was isolated from P388D.1 macrophages that had been incubated for 24 h in medium alone or in medium containing 500 units/mL IFN-γ and from the B cell line A.20. (D) Effect of LPS, PMA, and vitamin D on H mRNA expression in murine macrophages. Poly(A)+ RNA from equal amounts (200 μg) of total RNA was isolated from J774A.1 macrophages that had been stimulated with the indicated modulators.

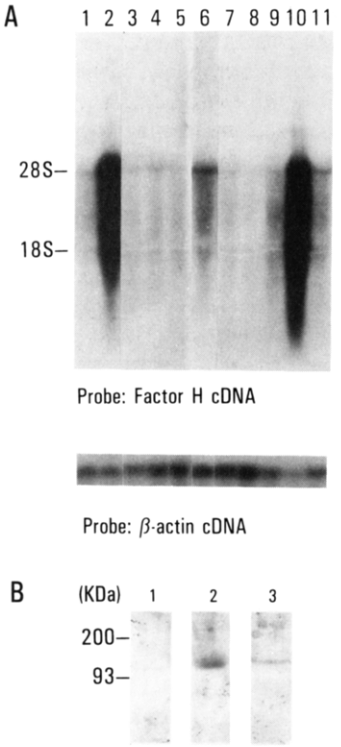

FIGURE 5: (A) Effect of modulators on H mRNA levels in L cells. Total RNA (20 μg) was isolated from L cells that had been incubated for 24 h in medium alone (lane 1) or in medium containing 10^{-7} M dexamethasone (lane 2), 10 ng/mL (lane 3), 50 ng/mL (lane 4), or 100 ng/mL (lane 5) LPS, 500 units/mL IFN-γ (lane 6), 100 ng/mL PMA (lane 7), 10 ng/mL vitamin D (lane 8), 500 units/mL IFN-γ + 10 ng/mL vitamin D (lane 9), 500 units/mL IFN-γ + 10^{-7} M dexamethasone (lane 10), and 500 units/mL IFN-γ + 50 ng/mL LPS (lane 11) and was subjected to Northern blot analysis with an H cDNA probe (top) or a β-actin probe (bottom). (B) Effect of dexamethasone and IFN-γ on H protein levels. Supernatants from cells corresponding to lanes 1, 2, and 6 from panel A were analyzed on a Western blot with an anti-H antibody. Lane 1, unstimulated cells; lane 2, cells stimulated with 10^{-7} M dexamethasone; lane 3, cells stimulated with 500 units/mL IFN-γ.

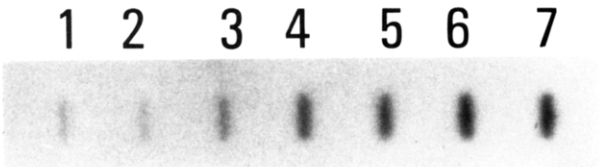

FIGURE 6: Dose response of IFN-γ for the increase of H mRNA level. L cells were incubated for 24 h in medium with or without increasing concentrations of IFN-γ. Total cellular RNA was isolated and 20 μg was analyzed in slot blots with H cDNA. Lane 1, unstimulated cells; lanes 2–7, cells stimulated with 1, 10, 100, 500, 1000, and 1500 units/mL IFN-γ, respectively.

mRNA Level. L cells were incubated alone or with increasing concentrations of IFN-γ ranging from 1 to 1500 units/mL for 24 h, and total RNA was analyzed by slot blot for H mRNA expression. Figure 6 shows the IFN-γ concentration dependence for the accumulation of H mRNA. A 3-fold increase is obtained when L cells are incubated with 10 units/mL IFN-γ. A maximal increase of 5.4-fold is obtained with 100 units/mL IFN-γ, and then the response plateaus.

Analysis of Dexamethasone Effects on H mRNA Levels. L cells were incubated alone or with increasing concentrations of dexamethasone, ranging from 10^{-12} to 10^{-5} M, for 24 h. Total RNA from each of the cell populations was isolated and analyzed for H mRNA expression. Figure 7 shows the dexamethasone concentration dependence for the accumulation

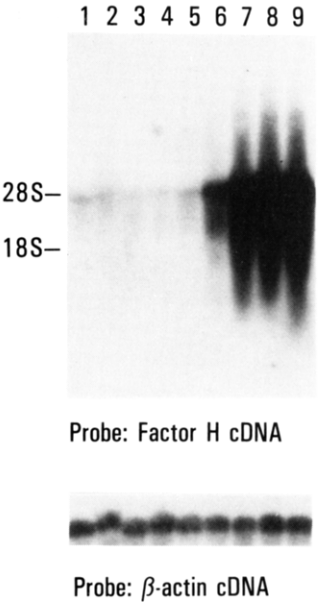

FIGURE 7: Dose response of dexamethasone for the increase of H mRNA level. L cells were incubated for 24 h in the absence (lane 1) or presence of increasing concentrations of dexamethasone. Total cellular RNA was isolated and Northern analysis was performed with 20 μg of RNA per lane. Lanes 2–9: 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M dexamethasone, respectively.

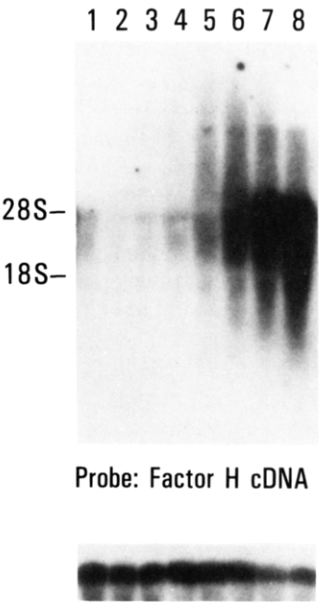

FIGURE 8: Time course of dexamethasone for the increase of H mRNA level. L cells were incubated with 10^{-7} M dexamethasone for increasing periods of time. Total cellular RNA was isolated and Northern analysis was performed with 20 μg of RNA per lane. Lane 1, unstimulated cells; lanes 2–8, cells stimulated for 10 min, 30 min, 1 h, 2 h, 4 h, 8 h, and 24 h, respectively.

of H mRNA. When as little as 10^{-12} M dexamethasone is used to stimulate L cells, a 2.3-fold increase in H mRNA levels is observed. These levels increase with higher doses of dexamethasone and eventually reach a plateau at 10^{-7} M dexamethasone.

The time course of dexamethasone stimulation was examined next. L cells were incubated with 10^{-7} M dexamethasone for various periods of time ranging from 0 to 24 h. The results shown in Figure 8 indicate that incubation of L cells with dexamethasone caused an increase in H mRNA that was

detectable as early as 30 min, with a 1.3-fold induction after hormone addition. The peak response was measured at 24 h, with an approximate 30-fold increase in RNA levels.

Discussion

The recent isolation of cosmid clones containing the genomic sequence for H and the determination of the structure of this gene have allowed a closer examination of its 5′ regulatory region. More than 3 kb of 5′ flanking DNA were sequenced and the genomic sequence from this region was used in S1 nuclease analysis of H mRNA transcription start sites in both liver and L cells (Figure 2). These results were confirmed by using RNA probes synthesized from this region. Two start sites were found in the liver, located 165 and 250 nucleotides upstream of the ATG start codon, and the former one was also found to be used in L cells. We have designated the G's located at these positions as the start site (Figure 1). However, the actual start site may be located 1–2 nucleotides on either side. The significance of two transcription start sites for H mRNA in the liver is unknown and, because of their proximity to one another, could not have been observed in Northern blots under ordinary conditions. We are currently examining the relative activity of these two promoters using the luciferase system.

One of the most important aspects of obtaining the nucleotide sequence of the 5′ flanking DNA region is to examine it for the presence of cis-acting regulatory or enhancer elements. Such enhancer elements have been associated with modulating the activity of proximal promoter elements (Dynan & Tjian, 1985). However, the identification of enhancer-type elements by comparative analysis is difficult because there are many types of enhancer sequences, their effect is not dependent on orientation, and they can be located at distances far from the transcription start site. Although consensus sequences for many enhancers exist, these sequences are generally short, less than 15 nucleotides. Thus, the probability of finding sequences that closely match any consensus enhancer sequence is high, and authenticity of any potential enhancer element requires in vitro or in vivo expression analysis. The examination of the 5′ terminal region of the H gene has revealed the presence of several sequences with high homology to elements thought to participate in regulating gene expression. In view of the good sequence conservation of some of these elements, it is likely that they may serve as transcriptional control sites of the H gene, especially the SV40 enhancer and the GRE. The former has been proven to be a real enhancer for human factor B gene transcription (Wu et al., 1987).

When RNA from murine liver is probed with H cDNA, four transcripts are identified of 4.4, 3.5, 2.8, and 1.8 kb, respectively. The 4.4-kb one corresponds to the authentic full-length H mRNA. This hybridization pattern differs from that seen in Northern blots of human liver, where two highly abundant species of 4.3 and 1.8 kb, respectively, and a much less abundant one of 1.2–1.5 kb are found, the largest of which represents the full-length human H mRNA (Schwaeble et al., 1987; Ripoche et al., 1987, 1988a). In humans, the 1.8-kb transcript apparently corresponds to a 5′ truncated form of H message, and the 1.2–1.5-kb transcripts are reactive only with 3′ H probes. Both transcripts seem to be translated in the liver, as two monoclonal anti-H antibodies, specific for the N-terminal and C-terminal regions of H, respectively, react with several plasma proteins of 40–50 kDa (Fontaine et al., 1989). In mice, the origin of the three additional smaller RNA species seems to be different, as they hybridize with a 3′ H cDNA probe but not with a 5′ H cDNA probe (Vik et al., 1989). Several cDNA clones have been isolated from a murine liver library that are highly homologous to yet distinct from H, and thus these smaller transcripts may be products of genes that are related to H, but not the result of alternative splicing. Very little was known about the tissue-specific expression of H mRNA in mouse. Northern blot analysis of murine kidney, spleen, and thymus RNA has revealed the presence of the 4.4-kb full-length H transcript (Figure 3). Similar studies will need to be done in humans in order to be able to compare the distribution patterns in both species. There have been several studies concerning H biosynthesis and mRNA expression by human cell lines (Malhotra & Sim, 1985; Brooimans et al., 1989), which have shown that H is expressed in skin fibroblasts, endothelial cells, the promonocyte cell line U937, and possibly peripheral blood monocytes. Neither of the macrophage cell lines examined in this study expressed H mRNA, nor could they be induced to express it. However, one of two liver cell lines and the fibroblast cell line L929 were both shown to express two forms of H mRNA, the 4.4 and the 1.8 kb, respectively (Figure 4). The full-length (4.4 kb) species was easily detected in Northern blot analysis while the 1.8-kb form was only demonstrated after longer exposure of the same blots, indicating that the latter is weakly expressed in both cell lines. This observation differs with results obtained with human fibroblasts, in which the two H transcripts are detected at similar levels (Katz & Strunk, 1988).

Fibroblasts are ubiquitous in most tissues. Therefore, the expression of H by these cells could provide an important source of this regulatory component during complement activation in tissues. Thus, L cells were examined for their capacity to be modulated for H mRNA expression by various external stimuli that might be present during an inflammatory response (Figure 5). The increase by IFN-γ is similar to that observed in human fibroblasts for H, where the combination of LPS and IFN-γ had the same effect as IFN-γ alone (Katz & Strunk, 1988). The lack of responsiveness to LPS is also in agreement with the fact that LPS induces an increase in the synthesis of proteins involved in the activation of the alternative pathway of complement but has no effect on the inhibitory factors (Reid & Porter, 1981). The maximal response in L cells to IFN-γ was reached at a dose of 100 units/mL (Figure 6), which is similar to that seen in human fibroblasts, where a maximal response was obtained at 100 units/mL (Katz & Strunk, 1988), and in human endothelial cells, where a maximal response in H mRNA induction was obtained at 100 units/mL (Ripoche et al., 1988b) or 200 units/mL (Brooimans et al., 1989).

Pursuant to the discovery of two GRE in the 5′ regulatory region of the H gene, the effect of dexamethasone on H mRNA levels in L cells was examined. The addition of this hormone to cell cultures led to a more than 30-fold increase in the levels of H mRNA (Figures 5, 7, and 8). The dose–response analysis of this hormone has shown that the maximal and half-maximal responses occur at concentrations similar to those required for other dexamethasone-mediated responses, including the induction of tyrosine aminotransferase (Gelehrter & McDonald, 1981) and of plasminogen activator–inhibitor mRNA (Heaton & Gelehrter, 1989), suggesting that the dexamethasone regulation of H mRNA in L cells is regulated by the glucocorticoid receptor. This possibility is enhanced by the presence of two GRE in the 5′ flanking region of the H gene. However, posttranscriptional alterations in the stability of H mRNA may also participate in the overall changes of H mRNA concentration that occur after dexamethasone incubation of the cells. To distinguish between these alternatives, further studies will need to be performed to measure

the rate of H transcription and mRNA stability.

Following our observations that IFN-γ and dexamethasone up-regulated the levels of H mRNA, we next examined the effects of these modulators on H protein production. Both of these stimuli increased the levels of the 150-kDa H protein in the supernatant of L-cell cultures, as determined by Western blotting (Figure 5B). Therefore, the increased levels of H mRNA caused by IFN-γ and dexamethasone result in a concomitant increase in H protein synthesis.

The immunosuppressive and antiinflammatory potencies of glucocorticoids have long been known and used in medical practice. Glucocorticoids reduce the number and influence the function of lymphocytes, monocytes, and eosinophils in peripheral blood (Nelson & Conn, 1980). Prolonged high doses result in decreased levels of immunoglobulins. Therefore, it appears that the immunosuppressive effect of steroid hormones is a multipathway one. This is the first evidence that changes in RNA and protein levels of a complement component can be induced by a steroid hormone. The fact that H is a negative regulator of the complement cascade, together with our results of its dramatic increase by dexamethasone, is in agreement with the known immunosuppressive effects of glucocorticoids. The remarkable increases in H mRNA levels after dexamethasone administration and the presence of these GRE in the promoter region of the gene led us to speculate that this gene could be a target for direct control by the glucocorticoid–receptor complex. However, the functional relevance of these conserved sites can only be established by functional assays. Taking into account the potential post-transcriptional events that may take place, we think that these changes in H mRNA concentrations might be caused at least in part by increased transcription of the H gene. With the 5′ terminal sequence of the H gene in hand and using the luciferase reporter gene, we have begun to identify the functionally relevant control sequences for H gene expression. The mechanisms through which dexamethasone acts to increase H mRNA level are also currently under investigation.

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