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Regulation of Amyloid A Gene Expression in Cultured Cells

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Serum amyloid A (SAA) proteins are secreted by mammalian liver in response to inflammatory stimuli. Both transcriptional and posttranscriptional mechanisms have been shown to regulate the 2,000-fold increase in SAA mRNA after injection of endotoxin into mice. We report here the characterization of a cell line derived from mouse liver (BNL) in which the expression of SAA3 mRNA is regulated. In this model, SAA3 mRNA accumulated in response to conditioned medium from the mouse macrophage P388D1 cell line with kinetics similar to that seen in mouse liver (C. A. Lowell, R. S. Stearman, and J. F. Morrow, J. Biol. Chem. 261:8453–8461, 1986). In in vitro nuclear transcription assays, the SAA3 gene was transcribed equally in induced and uninduced cells. In addition, in steady-state RNA studies treatment with conditioned medium allowed the cells to rapidly accumulate SAA3 mRNA without an apparent change in half-life. These observations suggest that conditioned medium contains a factor(s) that acts directly on hepatocytes to regulate SAA3 mRNA processing.

Serum amyloid A (SAA) is a family of proteins secreted by mammalian liver in response to infection and inflammation (20, 30). SAA-derived protein is the major constituent of tissue deposits that characterize the disease secondary amyloidosis, a disabling and often fatal complication of chronic infections and inflammatory diseases such as tuberculosis and rheumatoid arthritis, respectively (13, 22). SAA protein synthesis is part of a broad mammalian response to injury, termed the acute-phase response, in which the synthesis in liver of other serum proteins, including fibrinogen, haptoglobin, and C-reactive protein (4–6, 20), is induced by lipopolysaccharide (LPS) and secondary macrophage products such as interleukin-1 and tumor necrosis factor (3, 16, 21, 31).

Three distinct genes for BALB/c mouse SAA have been cloned, sequenced, and mapped to chromosome 7 (25, 37, 38, 40). The SAA1 and SAA2 genes correspond to serum apolipoproteins sequence by Hoffman et al. (18). A protein for the recently discovered third gene has yet to be identified (37). Morrow and colleagues have characterized the kinetics of expression of the three SAA genes in the mouse in response to treatment with LPS (25, 30, 36). They have shown that SAA mRNA levels in the liver rise over 2,000-fold by 12 h after LPS injection; each of the three genes makes an equal contribution of mRNA. This increase in mRNA is accompanied by a 1,000-fold increase in the serum concentration of SAA1 and SAA2 protein (28). In their studies they showed that the rate of transcription of the SAA genes increases at least 200-fold for each gene. They concluded that both transcriptional and posttranscriptional mechanisms must account for the large accumulation in liver of SAA mRNA after LPS induction.

A cultured cell system for the study of mouse SAA gene expression would be useful to establish what intracellular, extracellular, and genetic factors regulate SAA gene expression. Here we describe such a system in which SAA3 mRNA is regulated. A BALB/c neonatal liver (BNL) cell line accumulates SAA3 mRNA in response to LPS-conditioned medium (CM) of P388D1 cells, a mouse macrophagelike cell line. The kinetics of expression is similar to that seen in liver. The induction of SAA3 mRNA is cell specific and greater than 150-fold. We show by nuclear transcription and measurement of SAA3 mRNA half-life that in this system posttranscriptional mechanisms must be invoked to account for the accumulation of CM-induced SAA3 mRNA. This cultured cell system provides evidence for the existence of a factor(s) secreted by activated macrophages that specifically regulates SAA3 gene expression posttranscriptionally.

MATERIALS AND METHODS

Cell lines and conditioned medium. BNL CL-2 and P388D1 interleukin-1-secreting cells were obtained from the American Type Culture Collection. L cells were a gift of H. Weintraub. BNL cells were originally isolated by culturing primary neonatal cells in medium that selected for cells retaining urea cycle enzymes in an attempt to generate a cell line with a hepatocyte phenotype (33). BNL and L cells were grown in Dulbecco modified Eagle medium (DMEM; GIBCO Laboratories) supplemented with 10% fetal calf serum (Hyclone), 100 U of streptomycin and penicillin per ml, and 10 mM glutamine. P388D1 is a neoplastic mouse lymphoid cell line which has retained many of the phenotypic features of macrophages, including synthesis of esterase, endocytosis of latex beads, and the ability to secrete monokines in response to activation with LPS (19, 39). P388D1 cells were grown in RPMI 1640 medium (GIBCO) supplemented with 10% heat-inactivated (56°C, 30 min) fetal calf serum, 10 mM nonessential amino acids (GIBCO), 100 U of streptomycin and penicillin per ml, and 10 mM glutamate. BNL cells were grown to confluence and split 1:10 twice a week. Fresh cultures from frozen stocks were started every other month. CM was prepared by the method of Drysdale et al. (11) by replacing unconditioned medium of P388D1 cells at 80 to 90% confluence with fresh medium containing 20 μg of *E. coli* O111:B4 LPS prepared by the Westphal method (Sigma Chemical Co.) per ml. P388D1 medium was harvested at 12 h, centrifuged to remove cells, and stored in working samples at −70°C. P388D1 cell viability after LPS treatment, determined by exclusion of 0.04% trypan blue, was always greater than 95%. All cells were grown at 37°C in a 6% CO_2 humidified environment.

Induction of SAA mRNA in mice and BNL cells. Unless otherwise noted, induction refers to the treatment of BNL cells with P388D1 conditioned medium. Three days after

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plating (70 to 90% confluence), BNL medium was replaced with fresh supplemented DMEM containing 20% CM. Higher doses resulted in cell toxicity and death. BNL cells were harvested at stated times by removing the medium, washing with physiologic buffered saline at 0°C, scraping the dish with a rubber policeman, and washing in physiologic buffered saline after a low-speed centrifugation. SAA was induced in mice with an intraperitoneal injection of the same endotoxin (2 μg/g of body weight). Liver was homogenized with a Teflon motorized pestle, filtered through cheesecloth, and processed as described above for BNL cells.

RNA analyses. RNA was prepared by the method of Auffray and Rougeon (1), except that the samples were sonicated for 10 s and precipitated twice in 3 M $LiCl_2$–6 M urea. The DNA content of the RNA preparation measured by fluorometry was less than 0.05% after a second LiCl-urea precipitation. RNA was fractionated on 17% formaldehyde–1.2% agarose gels and transferred to nylon membranes (Zeta-Probe; Bio-Rad Laboratories). Nick translations with two ^{32}P-labeled nucleotides were performed as described previously (27). All radiolabeled DNA was gel purified from bacterial vector DNA. Hybridizations in 10% dextran sulfate and 1% sodium dodecyl sulfate and washing were done under standard conditions (27). All autoradiography was with Kodak XAR film with a Cronex enhancing screen exposed at −70°C. Quantitation was performed with an LKB laser densitometer. Poly(A)$^+$ RNA was isolated by using oligo(dT)-cellulose (New England Biolabs) by the method of Aviv and Leder (2). Actinomycin D (Sigma) was made freshly; cells were treated at a final concentration of 20 μg/ml for the indicated times. BNL cells were treated when stated with a freshly made solution of emetine (Sigma) at a final concentration of 100 μM. Dexamethasone (Sigma) was dissolved in physiologic buffered saline, and the concentration was determined spectrophotometrically. In vitro transcription in isolated nuclei and primer extension were performed essentially as described previously (15, 37). Hybridizations were carried out in 1% sodium dodecyl sulfate–0.5% nonfat dry milk–300 mM NaCl–250 μg of *E. coli* tRNA (Calbiochem) per ml–10 μm EDTA–1× Denhardt solution–10 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (Sigma) at 65°C for 48 h and washed at 70°C in 15 mM NaCl–1.5 mM sodium citrate in 0.1% sodium dodecyl sulfate. α-Amanitin (Boerhinger Mannheim Biochemicals) was used at a final concentration of 2 μg/ml, and heparin (Sigma) was used at 1 mg/ml.

DNA probes. pRS48 is a partial cDNA of SAA3 (36) capable of detecting SAA1, SAA2, and SAA3 mRNA. GAPDH is the chicken glyceraldehyde-3-phosphate dehydrogenase gene provided by D. Bentley. pH2B is a chicken genomic clone of histone H2B provided by P. Challoner. pSA415 is a 1-kilobase *Bam*HI-*Bam*HI subclone containing the first and second exons of SAA3. pAA2 is a 1-kilobase *Xba*I-*Xba*I fragment of SAA3 containing exons 3 and 4. pmγAc is a mouse γ-actin cDNA; pmβ5 is a mouse genomic clone of β-tubulin. Both were provided by D. Cleveland. pBX is a derivative of pBR322 with an *Xho*I linker inserted at approximately position 3000. pSP-275 is a partial cDNA of the mouse SAA1 gene that recognizes both SAA1 and SAA2 mRNA but does not hybridize with SAA3 mRNA under the above conditions. This probe was provided by R. Meek.

All plasmids were grown in *E. coli* HB101 or DH1 and purified on successive $CsCl_2$ gradients, and the concentration of DNA was determined fluorometrically (23).

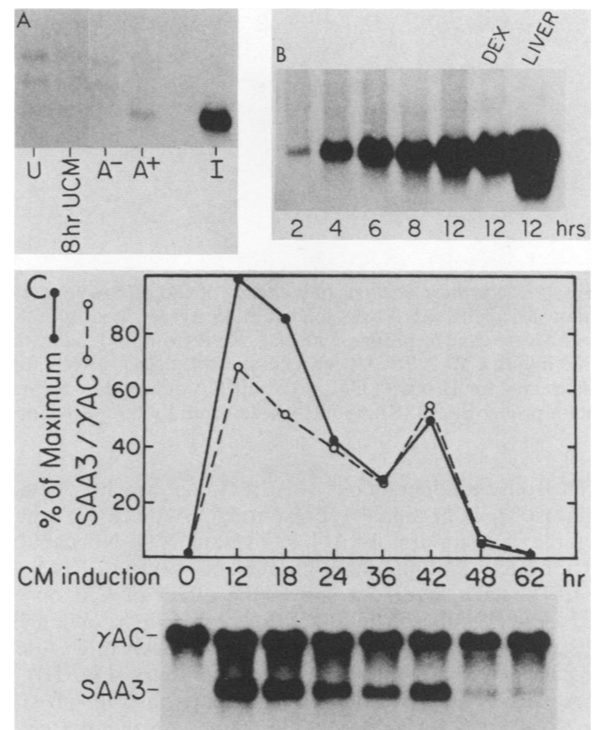

FIG. 1. (A) Northern analysis of BNL cell SAA mRNA. RNA was loaded at 10 μg per lane; pRS48 was used as a probe. Lanes: U, uninduced BNL RNA; 8 hr UCM, BNL cells treated with unconditioned P388D1 medium for 8 h; A$^-$, poly(A)$^-$ RNA from uninduced BNL cells; A$^+$, poly(A)$^+$ RNA from uninduced BNL cells; I, BNL cells induced for 12 h with 20% CM. Faint bands best visible in lane 1 represent 18S and 28S rRNA species determined by ethidium bromide staining. We estimate by hybridization with the γ-actin probe that oligo(dT) chromatography enriched for poly(A)$^+$ RNA approximately 10-fold (see Fig. 5C, lanes 3 and 4). (B) Northern analysis of the early time course of SAA mRNA accumulation in BNL cells treated with CM. RNA was loaded at 10 μg per lane except liver, in which 1 μg was loaded; pRS48 was used as a probe. Numbers below autoradiograph indicate length of treatment of BNL cells with CM in hours. DEX, Treatment of BNL cells with both CM and 1 μm dexamethasone in physiologic buffered saline; LIVER, 1 μg of BALB/c mouse liver harvested 12 h after an intraperitoneal injection of LPS (see Materials and Methods). (C) Northern analysis of the extended time course of SAA mRNA accumulation in BNL cells treated with CM. RNA was loaded at 10 μg per lane; pRS48 and pmγAc were used as probes. CM induction indicates length of treatment of BNL cells with CM in hours. SAA3 and γAC mRNA signals were quantitated by densitometry. The percentage of the maximum indicates the density of a given SAA signal relative to the maximum signal obtained at 12 h. SAA3/γAC ratio normalizes the SAA mRNA to the γ-actin mRNA signal at each time point. At least three separate experiments yielded the same result.

RESULTS

CM regulates SAA mRNA levels in BNL cells. BNL cells showed regulated expression of SAA mRNA when treated with CM. Figure 1A shows the Northern (RNA) blot analysis of RNA extracted from BNL cells and hybridized to the SAA3 cDNA pRS48, a probe capable of detecting SAA1, SAA2, and SAA3 mRNA species (26). No signal corresponding to SAA mRNA was detectable in 10 μg of total cellular RNA from uninduced BNL cells (lane U). Unconditioned P388D1 medium was unable to induce SAA mRNA (8 h in unconditioned medium). However, when total cellular

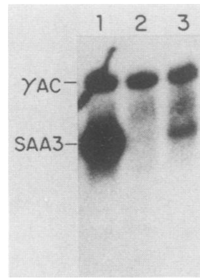

FIG. 2. Northern analysis of the effect of CM on SAA expression in mouse L cells. RNA was loaded at 10 μg per lane; pRS48 and pmγAc were used as probes. Lanes: 1, RNA from BNL cells treated for 12 h with CM; 2, RNA from uninduced L cells; 3, RNA from L cells treated for 12 h with CM. γAC, mRNA detected by the mouse γ actin probe; SAA3, SAA3 mRNA detected by the pRS48 probe.

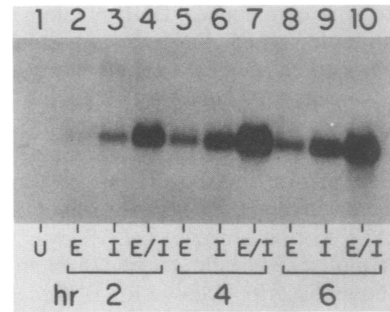

FIG. 3. Northern analysis of the effect of a protein synthesis inhibitor emetine on SAA mRNA expression in BNL cells. RNA was loaded at 10 μg per lane; pRS48 was used as a probe. Lanes: U, RNA from uninduced BNL cells; E, RNA from BNL cells treated with 100 μM emetine, I, RNA from BNL cells induced with CM; E/I, RNA from BNL cells pretreated with 100 μM emetine for 30 min and then treated with CM. Length of given treatment in hours is given below the autoradiograph.

RNA from uninduced cells was fractionated with oligo(dT)-cellulose, a faint signal representing SAA mRNA was detectable in 10 μg of poly(A)$^+$ RNA (lane A$^+$). No signal was detectable in a comparable amount of poly(A)$^-$ RNA (lane A$^-$). The lane labeled I shows the effect of CM on SAA mRNA accumulation in BNL cells after 12 h. When BNL cells were treated with CM, steady-state SAA mRNA levels increased over 100-fold within several hours (Fig. 1B). For SAA mRNA size and quantity comparison, Fig. 1B shows the SAA mRNA signal generated by 1 μg of total cellular RNA extracted from LPS-induced mouse liver at 12 h, a time at which each hepatocyte is estimated to contain 18,000 copies of total SAA mRNA (26). The SAA mRNA in induced BNL cells was identical in size (600 nucleotides) to that in induced mouse liver. Dexamethasone (1 μM), a known inducer of other acute-phase proteins (5), enhanced the induction by CM of SAA mRNA only minimally (Fig. 1B) and had no effect alone (data not shown). With a probe (pSP-275) specific for SAA1 and SAA2 mRNA, BNL cells expressed only the SAA3 mRNA (data not shown).

The kinetics of expression of SAA3 mRNA is shown in Fig. 1C. BNL cells were induced for up to 62 h with CM. By Northern analysis the peak concentration of SAA3 mRNA occurred at 12 h and returned to near-baseline levels by 48 h. RNA recognized by the mouse γ-actin cDNA probe showed little change in concentration over the course of induction. The actin mRNA thus served as a reference for equal loading of RNA and for poly(A)$^+$ enrichment for mRNA. We estimate that the steady-state levels of SAA3 mRNA are increased at least 150-fold by CM because we observed a 17-fold difference in SAA3 mRNA signal intensity between uninduced RNA enriched 10-fold by polyadenylation selection and total cellular RNA from induced BNL cells (Fig. 1A).

The cell specificity of CM-mediated SAA3 mRNA induction was examined by treating mouse L cells, a fibroblastlike cell line, in an identical manner with 20% CM. In Northern analysis (Fig. 2), CM had a markedly reduced ability to induce SAA3 mRNA in L cells (lane 3) compared with induction in BNL cells (lane 1). SAA3 mRNA was undetectable in 10 μg of total cellular RNA from uninduced L cells (lane 2). Other cell lines, including P388D1 cells themselves, showed minimal responsiveness to CM with respect to SAA gene expression (data not shown).

To test the necessity of new protein synthesis for the induction of SAA3 mRNA by CM, BNL cells were treated with CM or emetine, which inhibits protein synthesis by a mechanism of action similar to cycloheximide (24). Cells treated with both CM and emetine were pretreated for 30 min with emetine before CM induction. Emetine alone induced SAA3 mRNA (Fig. 3, lanes 2, 5, and 8) at a concentration of 100 μM, sufficient to inhibit protein synthesis greater than 90% (data not shown). When combined with CM, SAA3 mRNA is superinduced compared with either emetine or CM alone.

Transcription of the SAA3 gene in BNL cells is constitutive. To determine whether SAA3 mRNA induction by CM is controlled at the level of initiation of transcription, in vitro transcription of nuclei isolated from BNL cells was performed. In uninduced BNL cells the SAA3 gene was constitutively transcribed (Fig. 4A, lane 1, band d) at a rate comparable to that of the transcription of glyceraldehyde-3-phosphate dehydrogenase (band b), a housekeeping enzyme in the glycolytic pathway. When BNL cells were induced with fresh medium containing 20% CM, no change in tran-

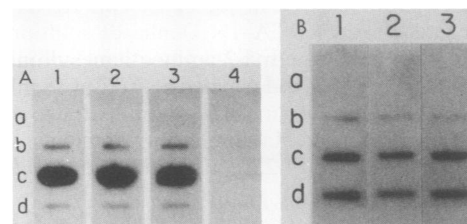

FIG. 4. (A) In vitro transcription with isolated BNL nuclei. Shown is an autoradiograph of the hybridization of nascent [^{32}P]RNA to 5 μg of the following DNA bound to mylon filters: a, a pBR322 derivative, pBX; b, GAPDH; c, mouse γ-actin cDNA, γAc; d, mouse SAA3 cDNA, pRS48 plasmid. Each filter was hybridized with 15 × 10^6 cpm of radiolabeled RNA synthesized in BNL nuclei isolated from the following (lanes): 1, uninduced cells; 2, cells treated with unconditioned medium for 4 h; 3, cells treated with CM for 4 h; 4, uninduced cells with 2 μg of α-amanatin per ml included in the in vitro transcription reaction. (B) Effects of emetine and heparin on in vitro transcription with isolated BNL nuclei. Shown is an autoradiograph of the hybridization of nascent [^{32}P]RNA to 5 μg of the following DNA bound to nylon filters: a, pBX; b, pγAc; c, pGAPDH; d, pRS48, the SAA3 cDNA. Each filter was hybridized with 20 × 10^6 cpm of radiolabeled RNA synthesized in BNL nuclei isolated from the following (lanes); 1, uninduced BNL cells; 2, BNL cells treated with 100 μM emetine for 2 h; 3, heparin (1 mg/ml) included in the in vitro reaction with nuclei from uninduced BNL cells.

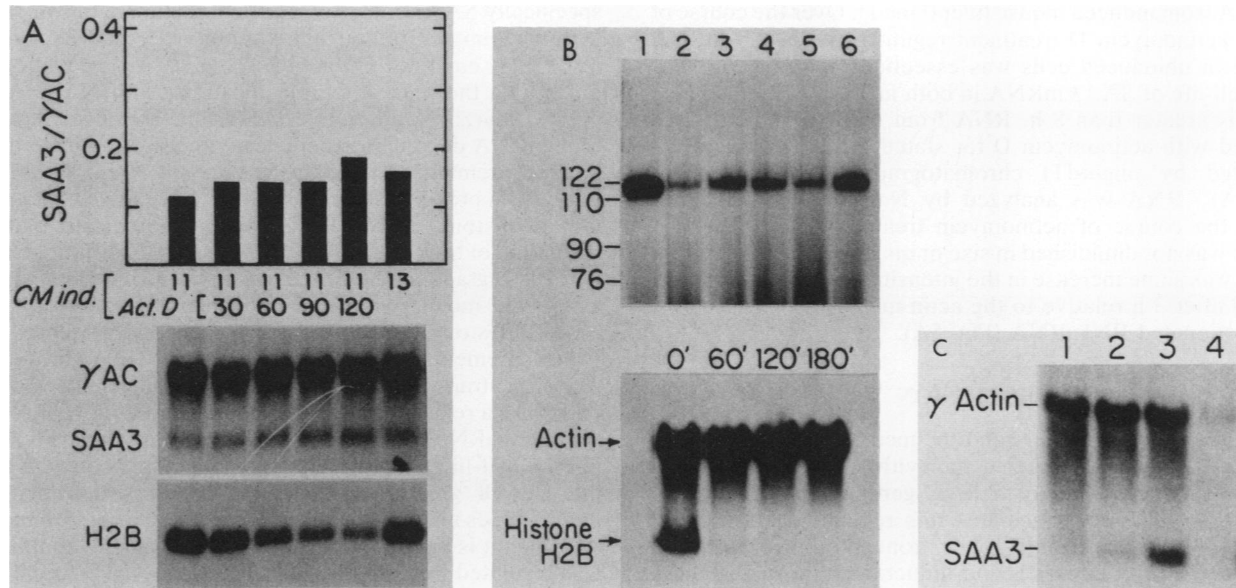

FIG. 5. (A) Measurements of half-lives of actin, SAA3, and histone H2B mRNA in BNL cells induced with CM. BNL cells were induced with CM (CM ind) for 11 h. Actinomycin D was added (20 μg/ml) to the culture medium and cells were harvested for RNA at 30, 60, 90, and 120 min thereafter; thus after 120 min of actinomycin D treatment, cells had been induced with CM for 13 h. A sample of BNL RNA from cells induced for 13 h is provided for comparison. γAC, mRNA detected by the γ-actin probe; H2B, RNA detected by the histone H2B probe; SAA3, RNA detected by pRS48. The relative ratio of the density of the SAA3 signal to the γ-actin RNA signal is shown above for each time point. (B) Measurements of half-lives of actin, SAA, and histone H2B mRNA in uninduced BNL cells treated with actinomycin D. BNL cells were treated with 20 μg of actinomycin D per ml and harvested at various times. Total cellular RNA (50 μg) was subjected to primer extension as described in Materials and Methods. Lanes: 1, 1 μg of 12-h LPS-induced mouse liver RNA; 2 through 5, BNL RNA prepared after 60, 120, and 180 min, respectively, of actinomycin treatment; 6, 10 μg of 12-h CM-induced BNL RNA. Total cellular RNA (10 μg) from the above samples was analyzed by Northern blotting for actin and histone H2B mRNA. Lanes contain RNA treated for 0, 60, 120, and 180 min with actinomycin D as indicated. (C) Northern analysis of SAA3 and actin oligo(dT)-selected mRNA from BNL cells. Poly(A)$^+$ BNL RNA (10 μg) was harvested at the times after treatment of BNL cells with actinomycin D. Lanes: 1, no actinomycin D; 2, 120 min of actinomycin D treatment; 3, 180 min of actinomycin D treatment; 4, 10 μg of total cellular RNA from uninduced BNL cells. After electrophoresis, the filter was hybridized successively with radiolabeled pRS48 and γ-actin DNA.

scription rates was observed (Fig. 4, lane 3, band d). This held true at 2, 4, and 6 h after induction (Fig. 4, lane 3; data not shown), times when SAA3 mRNA was easily detected by Northern analysis (Fig. 1B).

The constitutive transcription in vitro of the SAA3 gene is abolished by 2 μg of α-amanitin per ml, indicating that the signal observed in this assay is due to RNA polymerase II transcription (Fig. 4A, lane 4). To test whether attenuation of transcription might account for the low level of SAA3 mRNA in uninduced cells, we compared the transcription signal generated by the first two exons with that generated by the last two exons (see Materials and Methods). The intensities of the signals generated by these probes were not significantly different (data not shown). Because the sequence in these probes has not been fully determined, both radiolabeled uridine and guanosine triphosphates were employed in separate transcription assays to rule out a bias generated by a difference in nucleotide composition between the two regions, but again no difference between the 5′ and 3′ signals was observed (data not shown). Heparin, which removes most proteins other than already initiated RNA polymerase from the DNA template, also had no effect on the SAA3 transcription in vitro (Fig. 4B, lane 3). To determine whether the induction of SAA3 mRNA after treatment with emetine (Fig. 3) was due to an increase in transcription rates, we performed in vitro transcription with nuclei isolated at 0 and 2 h after treatment with 100 μM emetine. Emetine did not affect SAA3 transcription rates at 2 h (Fig. 4B, lanes 1 and 2), when SAA3 mRNA was detectable (Fig. 1B). We cannot exclude the possibility that transcription rates change only severalfold, because this magnitude of variability is inherent to the in vitro nuclear transcription assay.

Posttranscriptional regulation of the SAA3 gene. We measured the half-life of SAA3 mRNA by treating uninduced and CM-induced BNL cells with actinomycin D, an inhibitor of RNA polymerase. Cells were CM induced for 11 h and treated with actinomycin D, and SAA3 mRNA was assayed by Northern analysis at various times after treatment. SAA3 mRNA had a relatively long half-life in induced BNL cells (Fig. 5A). To control for the effectiveness of actinomycin D, we examined histone H2B mRNA from the same samples, an mRNA with a half-life on the order of 30 min (17). The half-life of histone H2B mRNA was approximately 30 min (Fig. 5A), indicating that actinomycin D effectively inhibited transcription. mRNA detected by the γ-actin probe, on the other hand, appears quite stable in agreement with a reported half-life of greater than 8 h (8). SAA3 mRNA has a stability comparable to that of actin mRNA. For comparison, we measured the half-life of SAA3 mRNA in uninduced cells. BNL cells were treated with actinomycin D in an identical manner, except that the cells were not induced with CM. Total cellular RNA was prepared at various times after treatment, 50 μg was annealed with a ^{32}P-labeled oligonucleotide primer complementary to SAA3 sequences from positions +86 to +116, and the primer was extended with reverse transcriptase. The expected 116-nucleotide extension product (Fig. 5B, lane 2) was identical in size to SAA3

mRNA from induced mouse liver (lane 1). Over the course of a 3-h actinomycin D treatment regimen, the SAA3 mRNA signal in uninduced cells was essentially unchanged. Thus the half-life of SAA3 mRNA in both induced and uninduced cells is greater than 8 h. RNA from uninduced BNL cells treated with actinomycin D for stated times was then fractionated by oligo(dT) chromatography, and 10 μg of poly(A)$^+$ RNA was analyzed by Northern blot analysis. Over the course of actinomycin treatment, SAA3 mRNA signal was not diminished in size or quantity (Fig. 5C); rather there was some increase in the intensity of the SAA3 mRNA signal after 3 h relative to the actin mRNA as was seen for unfractionated BNL RNA (Fig. 5A).

DISCUSSION

In an attempt to understand the mechanism of SAA gene expression, we characterized an in vitro cultured cell system that shows regulation of a SAA gene. The CM of LPS-treated P388D1 cells mediates this regulation. Uninduced BNL cells had extremely low concentrations of SAA3 mRNA (Fig. 1A and 5B), and induction by CM resulted a rapid 150-fold elevation of steady-state SAA3 mRNA (Fig. 1B). The kinetics of expression of SAA3 mRNA in induced BNL cells mimicked very closely that seen in mice after induction with LPS. In both cases the peak of accumulation occurs at 12 h and approaches an undetectable level by 48 h (Fig. 1C) (26). The effect of CM on BNL cells was specific for SAA3; mRNA from SAA1 and SAA2 expression was not detectable by Northern analysis. Thus, there may exist distinct pathways for the regulation of the SAA1, SAA2, and SAA3 genes. The effect of CM was also cell specific; only very low concentrations of SAA mRNA were detected in mouse fibroblastoid L cells after CM treatment (Fig. 2). That the regulation of SAA1 and SAA2 may be distinct from that of SAA3 and tissue specific is also indicated by the work of Meek and Benditt (29).

The results of in vitro nuclear transcription indicate that CM exerts no significant effect on the apparent rate of SAA3 transcription (Fig. 4A). In addition, heparin treatment of nuclei from untreated cells did not result in a more pronounced SAA3 transcription signal (Fig. 4B, lane 3). Thus it is unlikely that the failure to observe significant steady-state SAA3 mRNA before induction is due to inhibitory transcription elongation factor(s) and/or degradation of newly synthesized transcripts (9). The primer extension studies (Fig. 5B) indicate that SAA3 mRNA synthesis is initiated at the correct site in uninduced cells, excluding readthrough transcription initiated 5′ to the SAA3 promoter as the basis for the constitutive transcription signal (Fig. 4). In addition there is no evidence that induction represents the processing of a stable higher-molecular-weight RNA intermediate, because none was seen (Fig. 1A and B).

The mechanism by which CM exerts its effects was further investigated by determining whether new protein synthesis is required; it is not. In fact, emetine alone elevated SAA3 mRNA steady-state levels within 2 h, and induction with CM of BNL cells pretreated with emetine exhibited a supranormal response (Fig. 3, lanes 3, 6, and 9). In vitro transcription in nuclei isolated from emetine-treated BNL cells revealed no increase in the rate of SAA3 transcription (Fig. 4B), suggesting that emetine is not exerting its inductive effects by a mechanism involving an increase in SAA3 transcription, for example, by loss of a transcriptional repressor. One explanation for the effect of emetine is that the drug prevents the synthesis of a labile nuclease that degrades specifically SAA3 mRNA. CM might attenuate this nuclease activity. Another, although not mutually exclusive, possibility is that emetine prevents SAA mRNA degradation by preventing the release or translocation of mRNA on polysomes. Marzluff and coinvestigators have shown that histone mRNA degradation depends on nearly complete translation; premature termination or mRNA release by puromycin prevents the usual cell-cycle-dependent degradation of histone mRNA (14). Their data indicate that the regulation of the concentration of some mRNAs is controlled by their degradation by a mechanism involving ribosomes or associated molecules. We cannot distinguish among these possibilities or exclude other mechanisms at present.

Measurements of the half-life of mature SAA3 mRNA by using the transcriptional inhibitor actinomycin D indicate that it has a relatively long half-life, at least as long as that of the actin mRNA detected by our probe. We estimate a SAA3 mRNA half-life of greater than 8 h by comparison with actin mRNA. Of greater interest is the observation that the half-life does not change with CM treatment (Fig. 5B and C). Although it is possible that additional polyadenylation is the CM-regulated process by which SAA3 mRNA is stabilized, we would have expected, as others have observed, a lengthening in the size of the SAA3 mRNA in the course of induction (32). This was not observed (Fig. 1A and B). The observed histone H2B mRNA half-life in BNL cells agreed with what has been reported, indicating that actinomycin D is not prolonging mRNA half-lives in general. If CM had an effect on SAA3 mRNA half-life, it should be most evident at a time when SAA3 mRNA levels are rising compared with effects at a time when mRNA levels are falling. In fact, measurements of the SAA3 mRNA half-life at various times throughout the course of CM induction, including points when SAA3 mRNA levels are declining, yield similar half-life measurements. A preliminary approach to steady-state estimate of SAA3 mRNA half-life by [^{3}H]uridine labeling showed that in both induced and uninduced BNL cells [^{3}H]uridine incorporation rose throughout the 3-h course of labeling, corroborating the actinomycin D result that the half-life of SAA mRNA is relatively long (Rienhoff, unpublished data). It is impossible, however, by either of these methods to detect a prolongation of SAA3 mRNA half-life by CM from 8 h to an even longer time. We would therefore conclude that within the limits of these assays, CM has no demonstrable effect on SAA3 mRNA half-life.

If the rate of synthesis of SAA3 mRNA is equal for both induced and uninduced cells, as the in vitro nuclear transcription data would indicate, then CM must regulate some aspect of degradation: either a specific SAA3 mRNA degrading activity is attenuated by CM induction, permitting accumulation of SAA3 mRNA, or CM induction renders SAA3 mRNA inaccessible or poor substrate by virtue of modification for the specific degradation pathway. These models are not mutually exclusive. Heterogeneity of the population of SAA3 RNA molecules could account for these data. In one model a true molecular heterogeneity of SAA3 RNA would be due to differential processing of the nascent SAA3 transcript; two species of SAA RNA would be created—one highly labile form with a half-life on the order of less than 1 min and hence essentially unmeasurable and another species with a half-life of 8 h or more. Induction with CM would shift the equilibrium in favor of the processing pathway generating the more stable SAA RNA. Degradation in this model is therefore a deterministic process. An apparent but not true heterogeneity in the SAA RNAs would be evident if SAA RNA degradation were a stochastic process. In such a model

all SAA3 transcripts would be processed in a similar manner because of intrinsic identity, but the majority would be degraded by a temporally or spatially restricted SAA3-specific RNase activity. A small percentage of the SAA3 RNA, however, would escape destruction in uninduced cells, accounting for the weak signal we detected (Fig. 5B), whereas induction would increase the likelihood of either maturation to a stable species or of escape to an intracellular sanctuary or both. In either model all detectable mature SAA3 mRNAs, whether from induced or uninduced cells, would appear equally stable; the apparent half-lives would be similar, as we have observed.

Our data support the conclusion that posttranscriptional mechanisms involving RNA processing must account in large part for the accumulation of SAA3 mRNA in BNL cells in response to CM. The phenomenon of changes in mRNA levels without apparent changes in either the rate of transcription or in mRNA stability has been reported for class I major histocompatability mRNAs in adenovirus 12-transformed cells (34). Our observations are also consistent with the data reported for SAA gene expression in mice, in which posttranscriptional mechanisms account for the high level of SAA mRNA (26). Several reports indicate that sequences within transcribed portions of genes or secondary structures in mRNAs contribute to the regulation of steady-state mRNA levels (7, 10, 12, 35). Transfection studies in BNL cells with the SAA3 promoter fused to heterologous genes into BNL cells show essentially no regulation by CM, further supporting the hypothesis that the CM-responsive sequences in the SAA3 gene are 3′ to the transcription initiation site and not in the promoter (Rienhoff, submitted for publication). Experiments to identify CM-responsive sequences in the SAA3 gene are underway.

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