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Differential Glucocorticoid Enhancement of the Cytokine-Driven Transcriptional Activation of the Human Acute Phase Serum Amyloid A Genes, SAA1 and SAA2

Caroline F. Thorn and Alexander S. Whitehead

The human acute phase serum amyloid A (A-SAA) genes, SAA1 and SAA2, have a high degree of sequence identity that extends ~450 bp upstream of their transcription start sites. Each promoter contains analogously positioned functional binding sites for the transcription factors NF-κB and NF-IL6. In human HepG2 hepatoma cells transfected with SAA promoter luciferase reporter constructs, administration of IL-1 and IL-6, singly or in combination, induced SAA1 and SAA2 transcriptional readouts that were qualitatively indistinguishable. However, under induced conditions, the SAA2 promoter had a significant quantitative transcriptional advantage over the SAA1 promoter. The application of the synthetic glucocorticoid dexamethasone in the context of cytokine stimulation enhanced the transcriptional activity of the SAA1, but not the SAA2, promoter such that readout from the former became equivalent to that from the latter. A putative glucocorticoid response element (GRE) is present (between residues –208 and –194) only in the SAA1 gene; a similar sequence in the corresponding region of the SAA2 gene is disrupted by a nine-residue insertion. The SAA1 GRE was shown to be functionally active and the SAA2 disrupted GRE was shown to be functionally inactive in experiments using reporter constructs carrying SAA1 and SAA2 promoters that had been modified by site-specific mutagenesis. Quantitative analysis of transcript-specific RT-PCR products, derived from SAA1 and SAA2 mRNAs after treatment of HepG2 cells with cytokines in the presence or absence of dexamethasone, confirmed that the endogenous SAA1 gene has a cytokine-driven transcriptional disadvantage that is superseded by a marginal transcriptional advantage when glucocorticoids are present. The Journal of Immunology, 2002, 169: 399–406.

The acute phase response (APR) occurs in the hours immediately following an inflammatory stimulus such as infection or trauma (1). It is initiated, and subsequently maintained, modulated, and terminated, by the coordinated release of proinflammatory cytokines, anaphylatoxins, anti-inflammatory cytokines, cytokine antagonists, and glucocorticoids (2). During the APR, characteristic changes occur in the synthetic profile of the liver leading to altered regulation of a subset of products called acute phase proteins (APPs). These include positively regulated APPs such as acute phase serum amyloid A (A-SAA), C-reactive protein, complement C3 and C5, and negatively regulated APPs such as albumin (3). A-SAA is a major APP in that it is subject to quantitatively dramatic induction; at the peak of an APR it can be increased 1000-fold (i.e., levels exceeding those observed under normal physiological conditions by >1000-fold) (4).

Human A-SAA comprises two apolipoproteins, SAA1 and SAA2, the products of the SAA1 and SAA2 genes, which map to a cluster of related genes on chromosome 11p15.1 (5). The other members of the cluster are SAA3, a pseudogene (6), and SAA4, which encodes the constitutively expressed C-SAA (7, 8). In mouse, the Saa genes are arranged in a similar configuration on a region of distal chromosome 7 that has synteny with human chromosome 11p15 (9). The sequences of A-SAAs from vertebrate species that last shared a common ancestor hundreds of millions of years ago are remarkably conserved (10), indicating that these APPs have a fundamentally important protective function that has been retained via positive selection of critical structural motifs. The sequence similarities between the A-SAA genes within a given species are striking and extend beyond the coding regions to the mRNA untranslated regions (UTRs), introns, and upstream promoter elements, suggesting that they may have been subject to regular homogenizing events by a mechanism such as gene conversion (11). Based on the evolutionary considerations outlined above, and on direct experimental observations in vivo and in vitro, it has generally been assumed that the SAA1 and SAA2 genes are coordinately regulated and that their protein products play essentially identical roles in the APR. However, the apparent paradox of why higher mammals have at least two A-SAAs has thus far not been resolved.

During an APR the A-SAAs are expressed at high levels in liver and at more modest levels in many other tissues (12–15). A-SAAs are the circulating precursors of the insoluble cleavage product amyloid A that is deposited in major organs in secondary amyloidosis, a progressive and fatal disease that is an occasional consequence of chronic or episodic inflammatory conditions such as rheumatoid arthritis and leprosy (16). In addition to this well-documented role in the etiology of secondary amyloidosis, the A-SAAs may have other pathogenic actions: 1) they associate with HDL3 particles, replacing ApoA1 as the predominant apolipoprotein (17), and may therefore compromise reverse cholesterol transport and indirectly contribute to atherogenesis in those with chronic inflammatory conditions; 2) they are synthesized in the...
foam cells, smooth muscle cells, and endothelial cells of atherosclerotic plaques (18) and are chemotactic for monocytes and T cells (19, 20), suggesting that they may directly participate in lesion formation; and 3) they are produced in synovocytes and appear to function as autocrine inducers of collagenase (21), indicating a possible role in the etiology of degenerative joint diseases.

Given the known and presumptive clinical consequences of chronically high systemic concentrations of A-SAA and the sustained local synthesis of A-SAA, it is important to fully characterize the mechanisms whereby the synthesis of this archetypal major APP is induced, and subsequently controlled, under a variety of pro- and anti-inflammatory conditions. Over the past decade studies of SAA1 and SAA2 promoter-reporter constructs (22–24) have helped to define the parameters that govern the responsiveness of each of these genes to IL-1, TNF, and IL-6, alone and in combination, and have identified NF-κB and NF-IL6 transcription factor binding sites that facilitate responses of both genes to cytokine signaling that are qualitatively indistinguishable. However, no published studies to date have explored the mechanisms underlying the striking enhancement of cytokine-driven A-SAA mRNA and protein synthesis that is conferred by glucocorticoids both in vivo and in vitro (13–15, 25–29).

Materials and Methods

SAA promoter luciferase reporter constructs

Generation of the pGL2-SAA2pt construct, which contains 1.2 kb of the human SAA2 promoter upstream of a luciferase reporter gene, is described elsewhere (24). The pGL2-SAA1pt construct was generated as follows. The proximal 3.1 kb of genomic sequence upstream of the human SAA1 transcription start site plus the full 37 bases of 5′UTR was amplified by PCR from human genomic DNA (Roche, Indianapolis, IN) using forward and reverse primers, HSAAP1F (5′-GAATTCCGCGTTGGGACGGAAT ATACCTTATATTAGAA-3′) and HSAAPR (5′-GAATTCCTCGTGTGATCTGAGCTGCGG-3′), that incorporate MluI and Ncol restriction sites, respectively. The product was digested with MluI and Ncol and directionally cloned into a pGL2 vector that had been modified to include an Ncol site at the start of the luciferase coding sequence and contains the human SAA2 3′UTR (30). Constructs pGL2-SAA1[0.7] and pGL2-SAA1[0.25] containing 704 and 233 bases of promoter sequence, respectively, were generated from pGL2-SAA1pt by PCR using the forward primers 5′-GAATTCCGCGTTGGGACGGAAT ATACCTTATATTAGAA-3′ and 5′-GAATTCCTCGTGTGATCTGAGCTGCGG-3′, respectively, and the reverse primer HSAAPR. Constructs pGL2-SAA2[0.7] and pGL2-SAA2[0.25] containing 700 and 239 bases of promoter sequence, respectively, were generated from pGL2-SAA2pt by PCR using the forward primers 5′-TATAAACCGGCTTCTTATTAACGCCACACTCT-3′ and 5′-GAATT CACCGGGTATCAGCTACCTG-3′, respectively, and the reverse primer HSAAPR. The GREI construct was generated by PCR mutagenesis of pGL2-SAA1[0.7] using primers GREIF (5′-CAGCAAACTCTCTTGCTGCC-3′) and GREIR (5′-AGAGAGGTTCCTGTTGCTGG-3′). The GRED construct was generated by PCR mutagenesis of pGL2-SAA2[0.7] using primers GREDF (5′-CAAGGCCATCTCTGTTTCCTACAGTT-3′) and GREDR (5′-GGAACAAAGTGTCGCTTGGCAAGT-3′). The integrity of all constructs was verified by DNA sequencing.

Other plasmids

The Renilla transfection control plasmid is described elsewhere (31). The constitutive human glucocorticoid receptor (GR) expression plasmid, CMX-GR, was a gift of Dr. R. Evans (Salk Institute, La Jolla, CA) (32).

Cell culture and transient transfection

Human HepG2 hepatoma cells (American Type Culture Collection, Manassas, VA) were cultured in DMEM containing 10% FCS, gentamicin, sodium pyruvate, and nonessential amino acids (Life Technologies, Rockville, MD). Cells were seeded into 24-well plates 24 h before transfection using FuGENE (Roche) as previously described (33). Cells transfected with SAA promoter luciferase reporter constructs and Renilla control plasmid were incubated for 16–20 h before replacement of culture medium with fresh medium alone or fresh medium containing 10 ng/ml cytokines and/or dexamethasone and/or RU-486 (mifepristone). Each experiment was performed in triplicate. IL-1 was purchased from PeproTech (Rocky Hill, NJ). IL-6 was obtained from AstraZeneca (Wilmington, DE). Dexamethasone and RU-486 were from Sigma-Aldrich (St. Louis, MO).

Luciferase assays

Cells were harvested at various times posttreatment, washed in PBS, and resuspended in passive lysis buffer (Promega, Madison, WI). Lysates were assayed for luciferase and Renilla activity using the LXR and Stop and Glo reagents (Promega) in a dual-injection luminometer (Turner Designs, Sunnyvale, CA). The mean ratios of luciferase to Renilla activity for each treatment (performed in triplicate) were calculated. The fold induction for each treatment at each time point, together with the SDs, were calculated relative to the ratios derived from triplicate controls treated with medium only for the same length of time. Each experiment was performed at least three times. Statistical significance was measured by a t test.

RT-PCR

Total RNA was prepared by LiCl urea extraction (34) from HepG2 cells treated for 24 h under various experimental conditions. RT-PCR was conducted in a two-step process. cDNA was reverse transcribed from 3 μg of total cellular RNA in a 25-μl reaction containing oligo(dT) primer, RNasin RNase inhibitor, and Moloney murine leukemia virus reverse transcriptase (Promega) at 42°C for 1 h. PCR was performed using 2 μl of cDNA product in a 50-μl reaction containing 125 μM primers, 200 μM dNTPs (Amersham Pharmacia Biotech, Piscataway, NJ), 1× PCR buffer, 5 mM MgCl2, and AmpliTaq polymerase (PerkinElmer, Wellesley, MA). The forward primer was 5′-CAGACAATAATCTCCATGCT-3′; the reverse primer was an equal mix of 5′-TITTTTTCACCTCTAAGATTATTA TAGA-3′ and 5′-TITTTTTCACCTCTAAGATTATTAGA-3′. PCR conditions were as follows: 95°C for 5 min, followed by 25 cycles at 94°C for 20 s, 51°C for 1 min, and 72°C for 1 min, followed by 72°C for 5 min. Products were separated on 8% polyacrylamide gels at 50 V for 24 h, stained with ethidium bromide, and quantified by NIH Image.

Computer analysis

Transcription factor consensus binding site predictions were made using the Signalscan and TESS programs available from the Center for Bioinformatics (University of Pennsylvania, Philadelphia, PA) at http://www.cbi.upenn.edu. Sequence alignments were conducted using the ClustalW program (35) available at http://pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_clustalwan.html.

Results

Studies of A-SAA transcriptional activation in a range of species have identified a number of A-SAA promoter elements that are targets for critical transactivating factors including NF-κB, NF-IL6, SEF, YY-1, AP-2, SAF, and Sp-1 (36–41). Equivalent consensus binding sites for several of these factors are present in the human SAA1 and SAA2 promoters (Fig. 1). Of these, the NF-κB site (from bases –91 to –82) and the NF-IL6 site (from bases –184 to –171) of SAA2 have been shown by functional studies to be important for facilitating the response to IL-1 and IL-6, respectively (23). Glucocorticoids have been shown, both in vitro and in vivo, to enhance the cytokine-driven induction of A-SAA mRNA and protein (25–29). However, no studies to date have characterized the intrinsic genetic elements that govern the glucocorticoid-mediated enhancement of A-SAA synthesis.

Sequence alignment of the proximal promoters of SAA1 and SAA2

To explore the basis of the glucocorticoid enhancement of A-SAA synthesis we initially undertook a comprehensive computational analysis of the promoters of the SAA1 and SAA2 genes. Alignment of 0.7 kb of the SAA1 and SAA2 sequences immediately upstream of their respective transcription start sites, using the ClustalW program, revealed a very high degree of sequence identity (87%) in the proximal ~450 bp, upstream of which the sequences exhibit a markedly decreased level of identity and many regions of noncontiguity. The alignment of the highly conserved proximal promoter regions is depicted in Fig. 1. Within this alignment there are two
short noncontiguous regions that each mandate the introduction of a gap of more than one residue into one of the promoter sequences. These deletions are in \textit{SAA1} relative to positions 207 to 200 of \textit{SAA2} and in \textit{SAA2} relative to positions 43 to 40 of \textit{SAA1}.

Close visual inspection revealed a putative 15-bp glucocorticoid response element (GRE) consensus sequence (GGCACATCTTGGTTCC) (42) in \textit{SAA1} (from 208 to 194) that encompasses the first of these noncontiguous regions. These residues are also present in \textit{SAA2} (within the sequence from 213 to 190) but are disrupted by nine residues from 207 to 199 that have no counterparts in the corresponding location in \textit{SAA1} (i.e., between \textit{SAA1} residues 203 and 202).

To establish the extent to which the \textit{SAA1} and \textit{SAA2} promoters exhibit qualitatively and quantitatively similar responses to cytokines, and to determine whether the putative \textit{SAA1} GRE defined above is functionally active, various reporter constructs containing native and modified \textit{SAA1} and \textit{SAA2} promoters were tested for their responsiveness to cytokines and glucocorticoids in vitro.

\textit{Transcriptional regulation of the SAA1 and SAA2 promoters in vitro by cytokines and glucocorticoids}

HepG2 cells transfected with either of the A-SAA promoter luciferase reporter constructs, pGL2-SAA1pt or pGL2-SAA2pt, were treated with cytokines for 3, 6, 9, and 24 h. The \textit{SAA2} promoter was moderately induced by IL-1 alone or IL-6 alone and synergistically induced by the simultaneous addition of both cytokines (Fig. 2A). IL-1-driven readout increased from 3 to 24 h, whereas IL-6-driven readout was highest at 3 h and decreased through 24 h. The synergistic response to dual treatment with IL-1 plus IL-6 followed a kinetic profile similar to that observed for IL-6 alone. These results were all in accord with data previously reported by us (24). The \textit{SAA1} promoter exhibited transcription induction profiles in response to single and dual cytokine treatments that were qualitatively and kinetically similar to those of the \textit{SAA2} promoter (Fig. 2B). This is consistent with our a priori expectation that the analogously positioned NF-\textit{B} and NF-IL6 sites in the \textit{SAA1} and \textit{SAA2} promoters would mandate similar cytokine responses for each. However, \textit{SAA2} appears to have a considerable (2- to 3-fold) quantitative transcriptional advantage over \textit{SAA1} in response to all three cytokine treatments.

Treatment of transfected cells with dexamethasone alone had no effect on the \textit{SAA1} promoter. However, cotreatment of transfected cells with 50 nM dexamethasone enhanced the cytokine-driven induction of the \textit{SAA1} promoter 2-fold for all treatments at all time points (Fig. 2B). In contrast, dexamethasone had no measurable effect on \textit{SAA2} promoter activity under any of the test conditions (Fig. 2A). These data establish that the \textit{SAA1} and \textit{SAA2} genes respond differentially to glucocorticoids in the context of an ongoing cytokine-dependent transcriptional induction.
A construct containing only 235 bases of the SAA1 promoter (pGL2-SAA1[0.25]) retained all of the quantitative, qualitative, and kinetic aspects of cytokine responsiveness and glucocorticoid enhancement exhibited by constructs containing 3.1 kb of sequence upstream of the SAA1 transcription start site, suggesting that all of the critical control elements engaged by the most important inflammatory mediators are located in this short region (data not shown).

Dose-dependent glucocorticoid enhancement of cytokine-driven SAA1 promoter activity

To eliminate the trivial possibility that the nonresponse of both A-SAA promoters to dexamethasone alone, and of the SAA2 promoter to dexamethasone in the context of cytokine induction, was due to suboptimal dosing, a range of dexamethasone concentrations was tested. HepG2 cells transfected with either pGL2-SAA1pt or pGL2-SAA2pt were treated with 10, 50, 100, or 500 nM or 1 μM dexamethasone in the presence or absence of IL-1 plus IL-6 for 4 h. Neither promoter showed any response to dexamethasone alone, even at the highest dose used (data not shown). In contrast to the cytokine-driven transcriptional readout from the SAA2 promoter, which could not be enhanced by dexamethasone at any concentration, the SAA1 promoter exhibited a clear dose-dependent enhancement of transcriptional activity (data not shown). This suggests that the dexamethasone enhancement of cytokine-driven SAA1 promoter activity involves specific receptor-mediated events, most likely via GRs.

The enhancement of cytokine-driven SAA1 promoter transcriptional activity by glucocorticoids is GR dependent

To definitively establish that the enhancement of cytokine-dependent SAA1 promoter transcriptional activity by glucocorticoids is mediated by the GR, cytokine and dexamethasone treatments similar to those described above were conducted in the presence of the GR antagonist RU-486 (mifepristone). HepG2 cells transfected with pGL2-SAA1pt were treated with IL-1 plus IL-6, in the presence or absence of 100 nM dexamethasone and/or 10 nM RU-486 for 4 h (Fig. 3). The presence of RU-486 alone had no measurable effect on the level of cytokine-driven SAA1 promoter transcriptional activity. However, RU-486 completely blocked the capacity of dexamethasone to quantitatively enhance the induction of the SAA1 promoter by cytokines, limiting the transcriptional readout to that observed in transfected cells treated only with cytokines. This establishes that the GR is a requisite component in mediating the dexamethasone enhancement of cytokine-driven SAA1 transcriptional activity.

The effect of GR overexpression on the capacity of dexamethasone to modify SAA1 and SAA2 transcriptional activity

HepG2 cells have been reported to express only low levels of GR (43). To determine whether the nonresponse of both promoters to

![FIGURE 2](image-url)  
**FIGURE 2.** Time course of cytokine/dexamethasone induction of SAA1 and SAA2 promoter luciferase reporter constructs. HepG2 cells transfected with pGL2-SAA2pt (A) or pGL2-SAA1pt (B) luciferase reporter constructs were treated with medium only, dexamethasone (50 nM), IL-1 (10 ng/ml), IL-1 plus dexamethasone, IL-6 (10 ng/ml), IL-6 plus dexamethasone, IL-1 plus IL-6, or IL-1 plus IL-6 plus dexamethasone. Cells were harvested 3, 6, 9, and 24 h after treatment and relative luciferase values were calculated and compared with untreated controls. The difference between pGL2-SAA1pt luciferase reporter construct treated with IL-1 plus IL-6 and treated with IL-1 plus IL-6 plus dexamethasone was statistically significant at all time points (p ≤ 0.02).

![FIGURE 3](image-url)  
**FIGURE 3.** SAA1 glucocorticoid responsiveness is GR dependent. HepG2 cells were transfected with pGL2-SAA1pt and treated with 10 ng/ml IL-1 plus 10 ng/ml IL-6, alone and in the presence of 10 nM RU-486 and/or 100 nM dexamethasone. Cells were harvested 4 h after treatment and relative luciferase values were calculated and compared with untreated controls. The difference between treatment with cytokines plus dexamethasone and cytokines plus dexamethasone plus RU-486 was statistically significant (p = 0.05).
dexamethasone alone, and that of the SAA2 promoter to dexamethasone in the context of cytokine induction, is due to cellular GR levels that are below a functional threshold, SAA1 and SAA2 transcriptional readout was measured in HepG2 cells cotransfected with a constitutive GR expression construct after treatment with various combinations of cytokines and dexamethasone. The SAA1 and SAA2 promoters were unresponsive to dexamethasone alone in HepG2 cells cotransfected with a GR expression construct together with pGL2-SAA1pt or pGL2-SAA2pt (data not shown). However, in the context of cytokine induction of the SAA1 promoter, the dexamethasone enhancement of cytokine-driven transcriptional readout could be augmented by cotransfection with GR expression construct (data not shown), suggesting that the glucocorticoid signaling capacity of native HepG2 cells is not maximized with respect to engagement of the SAA1 promoter GRE. In contrast, cotransfection of GR expression vector could not bring about a dexamethasone-dependent enhancement of cytokine-driven transcriptional readout from the SAA2 promoter (data not shown), establishing that the SAA2 promoter is truly unresponsive to glucocorticoids.

Confirmation that the putative GRE in SAA1 is functional

Site-directed mutagenesis experiments were performed to determine whether the putative SAA1 GRE is functional and to exclude the possibility that subtle differences in genomic context, rather than intrinsic sequence differences in the putative SAA1 GRE and SAA2 disrupted GRE, mandate the differential dexamethasone responsiveness of the genes. Two modified constructs were generated: the GREI construct contains an SAA1 promoter with a nine-residue sequence (GCAAAACCTC) inserted into the GRE to form an SAA1-like disrupted GRE; the GRED construct contains an SAA2 promoter in which the same nine residues have been deleted to form an SAA2-like GRE (Fig. 4A). The GREI and GRED constructs each retained the basal and cytokine-driven levels of transcriptional activity that are characteristic of the unmodified parental promoters from which they were derived. However, the GREI construct had lost the capacity to respond to dexamethasone in the presence of cytokines, whereas the GRED construct had gained this property (Fig. 4B), thereby establishing that the SAA1 GRE is functional and is both necessary and sufficient to confer glucocorticoid responsiveness in the context of cytokine stimulation.

Differential transcriptional regulation of the endogenous human SAA1 and SAA2 genes in response to glucocorticoids

To determine whether the results obtained using the SAA1 and SAA2 promoter luciferase reporter constructs accurately reflect the regulation of the endogenous genes with respect to cytokines and glucocorticoids, we developed an RT-PCR method whereby the relative proportions of the transcription products of each gene could be directly compared. The SAA1 and SAA2 mRNAs, although highly similar (91% identical overall), differ significantly in the central region of their 3′UTRs; the SAA1 mRNA 3′UTR relative to that of the SAA2 mRNA 3′UTR has four deletions totaling 26 residues (Fig. 5A). We designed forward and reverse PCR primers, each of which can bind cDNA derived from either A-SAA mRNA (Fig. 5A). RT-PCR using these primers generates bands of 335 and 361 bp corresponding to products generated from the SAA1 and SAA2 mRNAs, respectively. In addition, the primers span intron 3 of each gene, thereby permitting products of amplification from contaminating genomic DNA to be identified. The ratio of 335- to 361-bp products, as determined by image analysis following resolution on 8% polyacrylamide gels, reflects the relative concentrations of cellular SAA1 and SAA2 mRNAs and serves as a surrogate measure of the relative transcriptional activation of the SAA1 and SAA2 promoters.

The above RT-PCR method was applied to total RNA from untreated and treated HepG2 cells. Products derived from SAA1 or SAA2 mRNA were not detected in untreated cells or following treatment with dexamethasone alone (Fig. 5B, lanes 1 and 2). However, the ratio of SAA1:SAA2 PCR product was ∼2.5 following treatment with IL-1 and IL-6 (Fig. 5B, lane 3), indicating that the endogenous SAA2 gene has a significant transcriptional advantage (i.e., ∼2.5-fold) when induced by cytokines in the absence of glucocorticoids. In contrast, the ratio of SAA1:SAA2 PCR products from cells treated with IL-1 and IL-6 plus dexamethasone was 5:4 (Fig. 5B, lane 4), indicating that the above cytokine-driven transcriptional advantage of the SAA2 promoter is superceded by a modest SAA1 transcriptional advantage in the presence of glucocorticoids. Thus, the responses of the endogenous genes to different combinations of proinflammatory mediators parallel those observed in experiments using isolated promoters to drive a quantifiable reporter, thereby establishing that the SAA1 GRE is functional when in its native genomic and cellular environment.

Discussion

The human SAA1 and SAA2 genes are highly conserved and consequently have been considered to be coordinately regulated during the APR. In this paper we present evidence that glucocorticoids are able to enhance the transcriptional activity of the SAA1 gene,
FIGURE 5. Response of the endogenous human SAA1 and SAA2 genes to cytokines and dexamethasone. A, Alignment of the 335-bp SAA1 and 361-bp SAA2 RT-PCR product sequences. The numbering is in relation to the full mRNA sequence. The intron-exon boundary is marked with a vertical line; amplification from genomic DNA would generate a product that incorporates sequence encompassing the 384-bp (SAA1) or 394-bp (SAA2) intron at this position. The 3’ UTRs are underlined twice and the primer sequences are underlined once and the primer sequences are underlined twice. Diagonal lines represent 121 bases of aligned exon 4 coding sequences that contain no gaps. Dashes represent regions of the SAA2 3’ UTR that have no counterpart in the SAA1 3’ UTR. B, HepG2 cells were treated with medium only, 100 nM dexamethasone, 10 ng/ml IL-1 plus 10 ng/ml IL-6, or IL-1 plus IL-6 plus dexamethasone for 24 h. RNA was extracted, reverse transcribed, and amplified as described. PCR products were separated by 8% PAGE.

but not the SAA2 gene, via a GR-dependent targeting of a GRE that is functional only in the former, and that this capability is only realized in HepG2 hepatoma cells in the context of concomitant signaling by proinflammatory cytokines.

Our data from experiments using promoter reporter constructs strongly indicate that in HepG2 cells glucocorticoids cannot contribute to enhanced SAA1 transcription in the absence of cytokines. Indeed, even under experimental conditions in which augmentation of HepG2 intracellular levels of GR, a critical component of the glucocorticoid signaling pathway, was combined with excess glucocorticoid there was no induction of transcription in the absence of cytokines. These in vitro findings in a hepatic cell line are in accord with the reported in vivo observation that patients who were undergoing long-term glucocorticoid therapy but had no overt inflammation did not elevate A-SAA protein concentrations (28). In contrast, patients who were receiving long-term glucocorticoid therapy and subsequently contracted an infectious disease had A-SAA protein concentrations that were markedly increased relative to those seen in patients with an infectious disease who had not been receiving glucocorticoids (28). Furthermore, Yamada et al. (29) observed significantly higher A-SAA/C-reactive protein ratios in rheumatoid arthritis patients treated with glucocorticoids than in those treated with other disease-modifying anti-rheumatic drugs. The published studies outlined above suggest that glucocorticoids alone have no effect on hepatic A-SAA expression, but that under conditions in which there is an underlying inflammation, and hence ongoing cytokine signaling, hepatic A-SAA expression is greatly enhanced by the presence of glucocorticoids.

Up-regulation of hepatic A-SAA protein synthesis during the APR involves both cytokine- and glucocorticoid-mediated effects. During cytokine-driven induction the SAA1 and SAA2 promoters are both engaged by the transcription factors NF-κB and NF-IL6, with SAA2 having a transcriptional advantage over SAA1. When glucocorticoids are also present, the SAA1 promoter, but not the SAA2 promoter, is additionally engaged by GR, the net result of which is to enhance SAA1 transcriptional activity to a level similar to that of SAA2. We hypothesize the presence of a repressor that constitutively binds to the A-SAA promoters in untreated HepG2 cells such that access to the SAA1 GRE is physically blocked. We further hypothesize that the repressor can be displaced by cytokine-driven NF-κB activation and engagement of the NF-κB sites in the A-SAA promoters, thereby exposing the SAA1 GRE and permitting GR binding and transcriptional enhancement. A similar mechanism is evident in the rat SAA1 promoter in which YY-1-dependent transcriptional repression is overcome following its displacement by the binding of activated NF-κB to an adjacent target site (37). However, the possible involvement of GR as a transcriptional enhancer has not been explored in the SAA genes of the rat or any other species to date.

The data presented in this paper are derived from experiments using cultured hepatoma cells and reflect the regulatory criteria that govern transcription of the SAA1 and SAA2 genes in the liver. Most A-SAA protein in the circulation is liver derived, and its increased synthesis during an APR is achieved largely through increased transcription (4), with only a minor contribution from posttranscriptional mechanisms (30). Therefore, the transcriptional hypothesis proposed above may be directly relevant to circulating A-SAA protein concentrations. Thus, the ratios of SAA1 and SAA2 proteins may change over time with a bias strongly in favor of SAA2 in the early APR giving way to increasing relative amounts of SAA1 after the systemic release of glucocorticoids.
Furthermore, the absolute concentrations of each of the A-SAAs during chronic inflammation may depend on the nature of the underlying disease and thus the mix of pro- and anti-inflammatory mediators that are present. The introduction of anti-inflammatory steroid therapy may further modify the ratio of SAA1 and SAA2 proteins depending on the type and therapeutic dose of synthetic glucocorticoids used. Such changes in the ratios of SAA1 and SAA2 may have important clinical as well as biological implications, especially given that SAA1 has been reported to be the predominant isof orm found in human secondary amyloidosis (44).

Many nonhepatic cell types including monocytes, endothelial cells, epithelial cells, and smooth muscle cells have been shown to express A-SAA when stimulated with cytokines and glucocorticoids. There have been reports that in some of these, namely THP-1 monocytes, KB oral epithelial cells, and primary aortic smooth muscle cells, A-SAA synthesis can be induced by dexamethasone alone (13, 45, 46). This suggests that glucocorticoids may be the predominant inflammatory modulator with respect to A-SAA synthesis in some local tissues and further leads to the speculation that in such locations A-SAA synthesis may be restricted to, or heavily biased toward, SAA1. As alluded to above, the differential effect of glucocorticoids on SAA1 and SAA2 gene expression may provide the means to modulate the relative proportions of their products such that the protective role(s) of A-SAA is maximized locally as well as systemically.

Understanding the shared and divergent aspects of the transcriptional regulation of SAA1 and SAA2 has important implications for future work aimed at devising therapeutic strategies to specifically down-regulate A-SAA synthesis in secondary amyloidosis and other clinical conditions in which this APP is pathogenic (47). In addition, such understanding will facilitate the rational design of modified A-SAA promoters in our ongoing studies to optimize them for use as control elements to drive the production of anti-inflammatory agents in gene therapy approaches to the treatment of localized inflammatory diseases (47).

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