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Inflammation-induced recombinant protein expression *in vivo* using promoters from acute-phase protein genes

(lipopolysaccharide/sepsis/gene therapy/adenovirus vector)

ALAN W. VARLEY*, MARK G. COULTHARD†, ROBERT S. MEIDELL*, ROBERT D. GERARD*‡, and ROBERT S. MUNFORD*§

Departments of *Internal Medicine, †Pediatrics, ‡Biochemistry, and §Microbiology, University of Texas Southwestern Medical Center, Dallas, TX 75235

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ABSTRACT **We report that promoters for two murine acute-phase protein (APP) genes, complement factor 3 (C3) and serum amyloid A3 (SAA3), can increase recombinant protein expression in response to inflammatory stimuli *in vivo*. To deliver APP promoter–luciferase reporter gene constructs to the liver, where most endogenous APP synthesis occurs, we introduced them into a nonreplicating adenovirus vector and injected the purified viruses intravenously into mice. When compared with the low levels of basal luciferase expression observed prior to inflammatory challenge, markedly increased expression from the C3 promoter was detected in liver in response to both lipopolysaccharide (LPS) and turpentine, and lower-level inducible expression was also found in lung. In contrast, expression from the SAA3 promoter was found only in liver and was much more responsive to LPS than to turpentine. After LPS challenge, hepatic luciferase expression increased rapidly and in proportion to the LPS dose. Use of cytokine-inducible promoters in gene transfer vectors may make it possible to produce antiinflammatory proteins *in vivo* in direct relationship to the intensity and duration of an individual's inflammatory response. By providing endogenously controlled production of recombinant antiinflammatory proteins, this approach might limit the severity of the inflammatory response without interfering with the beneficial components of host defense and immunity.**

Most gene-therapy vectors in current use rely on exogenous viral promoters for expression of recombinant proteins *in vivo*. The simian virus 40, Rous sarcoma virus, and cytomegalovirus (CMV) early promoters are active in a wide range of tissues, often drive high-level constitutive expression, and do not require specific inducing signals. To meet particular therapeutic goals, however, it may prove important to restrict expression of a recombinant gene to certain tissues or cells and to vary expression levels in response to physiological conditions. In such circumstances, gene transfer using inducible promoters may have significant advantages. Inducible promoters have been shown *in vitro* to depend on tissue-specific transcription factors for expression, to vary widely in their level of activity, and to be regulated by extracellular signaling molecules (1). Promoters that respond to appropriate physiological signals may permit autoregulation of drug dose by natural homeostatic mechanisms.

A case in point is severe systemic inflammation. Although inflammatory cytokines [e.g., tumor necrosis factor and interleukin 1 (IL-1)] are critical components of the host defense against invading microbes, high concentrations of these and other cytokines may be deleterious to the host, contributing to organ failure, shock, and death. Unfortunately, the results of several clinical trials indicate that determining the optimal dose and timing of exogenously administered anticytokine proteins in septic patients is very difficult (2). None of these drugs has improved survival. Indeed, when administered in large doses to patients or produced in large amounts by gene transfer vectors in mice (2, 3), some cytokine antagonists have had deleterious effects, including increased mortality and profound immunosuppression. A better method for dosing these potentially beneficial drugs is needed.

The promoters for acute-phase protein (APP) genes are particularly attractive candidates for regulating recombinant anticytokine production. In response to inflammatory stimuli, transcription of APP genes may increase manyfold, generally in proportion to the severity of the inflammatory stimulus (4). In addition, individual APP promoters differ with respect to basal transcription level, activating stimuli, and degree of inflammation-stimulated induction (4). It may therefore be possible to construct gene transfer vectors that differ in their responses to inflammatory stimuli *in vivo*.

To test this possibility, we produced reporter constructs that contained the firefly luciferase gene controlled by the complement factor 3 (C3) and serum amyloid A3 (SAA3) gene promoters. Since intravenously injected adenoviruses infect hepatocytes, the site of most endogenous APP synthesis, we used nonreplicating adenovirus vectors to deliver the APP promoter–luciferase reporter gene constructs *in vivo*. We found that both promoters were positively regulated by inflammatory stimuli, yet they exhibited distinctive stimulus- and tissue-specific response patterns. Inducible promoters such as these may make it possible to produce recombinant antiinflammatory proteins *in vivo* in direct proportion to the intensity and nature of an individual's inflammatory response.

EXPERIMENTAL PROCEDURES

Cell Lines and Media. Hep G2 human hepatoma cells (ATCC HB 8065) were cultured in Dulbecco's modified Eagle's medium (DMEM; Mediatech, Washington, DC) containing 10% heat-inactivated, low-endotoxin fetal bovine serum (HyClone), 2 mM glutamine, penicillin (50 units/ml) and streptomycin (50 μg/ml). When used to culture 293 human embryonic kidney cells (ATCC CRL 1573), the medium was further supplemented with amphotericin B (0.25 μg/ml; JRH Biosciences, Lenexa, KS). Cytokine-rich conditioned medium (CM) was prepared from the culture supernatant of adherent human peripheral blood monocytes incubated overnight in RPMI 1640 (Mediatech) containing 7% fetal bovine serum, 1% human serum, lipopolysaccharide (LPS, 1 μg/ml, from *Escherichia coli* LCD25), and glutamine, penicillin, and streptomycin as indicated above. Cells were removed by a brief centrifugation, and the supernatants were filtered (0.22 μm)

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Abbreviations: APP, acute-phase protein; C3, complement factor 3; CM, conditioned medium; CMV, cytomegalovirus; IL, interleukin; LPS, lipopolysaccharide; moi, multiplicity of infection; pfu, plaque-forming unit(s); SAA3, serum amyloid A3; SAP, serum amyloid P.

and stored at −70°C until needed. If not otherwise indicated, reagents were purchased from Sigma.

C3 and SAA3 Reporter Constructs. Regulatory regions of the murine C3 and SAA3 gene promoters were amplified from mouse genomic DNA by PCR. The C3 promoter was amplified with oligonucleotide primers 5′-AGG-ATC-GAT-AAT-GCA-ATG-CCA-AAT-GTG-3′ and 5′-TTT-TGG-ATC-CAA-AAA-GGT-GGA-AGG-AAT-GAA-3′ (Molecular Cardiology Oligonucleotide Synthesis Facility of the University of Texas Southwestern Medical Center) flanking nt −397 to +48 (5). The SAA3 promoter was amplified with oligonucleotide primers 5′-CTC-ATC-GAT-ATC-CCA-TGA-TTT-ATC-ACA-C-3′ and 5′-TTT-TGG-ATG-CGG-GAC-CCC-AGG-TGA-GTG-G-3′, flanking nt −306 to +33 (6). The resulting PCR products were cloned with the TA cloning system (Invitrogen) to generate plasmids pCRII-C3 and pCRII-SAA3. The DNA sequences of both promoters were determined by M13 single-stranded sequencing with Sequenase version 2 (United States Biochemical). The SAA3 promoter was subcloned as a *Not* I–*Bam*HI fragment from pCRII-SAA3 into the polylinker upstream of luciferase in pBstLuc (Stephen Johnston, this institution) to generate pBstLuc-SAA3. Similarly, the C3 promoter was subcloned between the *Not* I and *Spe* I sites of pBstLuc to generate pBstLuc-C3.

Recombinant Adenoviruses. The inserts from pBstLuc-C3 and pBstLuc-SAA3 were cloned as *Eco*ICRI fragments into the *Sma* I site of pUC18 to obtain flanking *Sal* I and *Acc*65I sites. The inserts were then moved into the *Sal* I and *Acc*65I sites of pAC.ESHRpL(−) (7) to generate pAC-C3-Luc and pAC-SAA3-Luc. Human 293 cells (8) were then cotransfected with 10 μg of one of these plasmids and 5 μg of *Xba* I-digested viral DNA prepared from a recombinant adenovirus derived from Ad5dl309 (7, 9), by the calcium phosphate method with a glycerol shock to boost transfection efficiency. Recombinant adenoviruses were subjected to three cycles of plaque isolation and screening to ensure purity. Ad.CMV-Luc (containing the CMV early promoter) was prepared as described (10), and Ad.No-Luc (promoterless luciferase gene) was the gift of Michael McPhaul (this institution). Recombinant adenoviruses were grown to high titer in 293 cells, harvested, purified over a discontinuous CsCl gradient, and desalted on a Sepharose CL-4B (Pharmacia) column as described (7). After the addition of low-endotoxin bovine serum albumin (10 μg/ml), aliquots were quick frozen and stored at −70°C until used. The virus titer was then determined by plaque assay on 293 cell monolayers (11).

Promoter Analysis in Hep G2 cells. Hep G2 cells from a freshly confluent 10-cm plate were distributed in six-well plates (2×10^6 cells per well) and incubated overnight. The next morning, the growth medium was replaced with 1 ml of DMEM containing 2% fetal bovine serum and a recombinant adenovirus [multiplicity of infection (moi), 0.5–5], and incubation was continued for 90 min. The medium containing the virus was then aspirated, fresh growth medium was added, and the plates were returned to the incubator. Twenty-four hours later, cells were trypsinized in 1.2 ml and split into an equal volume of either whole growth medium or a 1:3 mixture of CM and whole growth medium (1:8 final dilution of CM). Cells were harvested 18 hr later by incubation for 20 min at room temperature in 200 μl of lysis buffer [PBS (137 mM NaCl/3 mM KCl/10 mM Na_2HPO_4/2 mM KH_2PO_4)/0.2% Triton X-100/2.5 mM EDTA/0.1 mM phenylmethylsulfonyl fluoride with aprotinin at 0.5 μg/ml and leupeptin at 0.5 μg/ml], transferred to a microcentrifuge tube, and clarified by brief centrifugation. The clarified lysates were either assayed immediately or stored at −70°C. Two to four separate infections were performed per experiment. Experiments were repeated at least once.

***In Vivo* Experiments.** Protocols for animal experiments were approved by the University of Texas Southwestern Institutional Review Board for Animal Research. Specific pathogen-free, male ICR mice (Harlan Laboratories, Indianapolis) weighing 20–24 g were housed in the institutional animal facility, fed standard mouse chow *ad libitum*, and used for experiments within 1 week of arrival. Each mouse was injected via its tail vein with adenovirus diluted in 0.25 ml of 0.9% NaCl. Three days later, mice in different groups received 0.9% NaCl (0.1 ml, s.c.), steam-distilled turpentine (0.1 ml s.c.), or *E. coli* O127:B8 LPS (Sigma; desired dose in 0.2 ml, i.p.). Eighteen to 24 hr later the mice were anesthetized (1 mg of pentobarbital, i.p.), bled from the retroorbital plexus, and killed by cervical dislocation. Organs were removed, weighed, and suspended in chilled lysis buffer (see above). The ice-cold tissues were then homogenized (Tissue Tearer; Biospec Products, Bartlesville, OK) or sonicated (2 min, constant cycle, Sonifier 450; Branson).

Assays. Luciferase activity was quantitated in cell lysates and organ homogenates as described (12), by use of an Optocomp II luminometer (MGM Instruments, Hamden, CT). Background light emission was subtracted from the raw light units (ILU, integrated light units—a dimensionless machine function) and the results were normalized to protein concentration (Coomassie blue G250 assay; Pierce) or tissue weight. Serum amyloid P (SAP) protein was measured by rocket immunoelectrophoresis (13, 14) using rabbit anti-SAP antiserum and three dilutions of commercial SAP (both from Calbiochem) as standards for each gel. IL-6 was measured by ELISA (PerSeptive, Boston).

Statistics. Differences between groups were analyzed by ANOVA (KWIKSTAT, version 4; Texasoft, Dallas). Differences with $P \leq 0.05$ were considered significant.

RESULTS

Cloning the Murine C3 and SAA3 Promoters. The regions of the C3 and SAA3 promoters responsible for induction of transcription in response to cytokines have been identified (5, 15). We cloned these regions by PCR and verified their structure by DNA sequencing. The sequence of our C3 promoter clone was identical to that in the GenBank database. However, our SAA3 promoter clone differed from the GenBank sequence at four positions: a C → G transversion at nt −117; a T → A transversion at nt −121; and two single base-pair deletions at nt −147 and −171. According to a SITES (32) analysis on our SAA3 sequence using the transcription factor database (32) (release 7.3), none of these changes affected potential binding sites for factors known to regulate APP genes.

Analysis of Promoter Activity in Hep G2 Cells. To characterize the reporter constructs functionally, we infected Hep G2 human hepatoma cells with recombinant adenoviruses containing chimeric promoter–luciferase constructs and measured luciferase activity before and after stimulation with cytokine-rich CM. No luciferase activity was detected in uninfected cells or in cells infected with an adenovirus containing a promoterless luciferase gene (Ad.No-Luc) (16). In contrast, cells infected with adenoviruses containing the C3– or SAA3–luciferase constructs exhibited significant levels of induction by CM (Table 1). Although the basal levels of luciferase activity in cells infected with these constructs were indistinguishable, activity in cells induced with CM was slightly higher in Ad.SAA3-Luc-infected cells (19,790 light units/μg of protein) than in cells infected with Ad.C3-Luc (4610 light units/μg of protein). The constitutive CMV promoter showed high basal expression and significant inducibility (Table 1). Induction from this promoter varied from 3- to 10-fold over a range of Ad.CMV-Luc concentrations (moi from 0.1 to 10), with optimal induction occurring at an moi of 0.5–1 (data not shown).

Analysis of Promoter Activity in Mice. To determine whether the cytokine induction observed *in vitro* could be

Table 1. Induction of luciferase reporter constructs *in vitro*

Adenovirus construct	Luciferase activity, light units/μg of cell protein: Control medium	Cytokine-rich medium	Fold induction
Ad.No-Luc	2	6	3
Ad.CMV-Luc	2220	12,680	6
Ad.C3-Luc	180	4,610	26
Ad.SAA3-Luc	570	19,790	35

Hep G2 cells were infected at a moi of 5 and incubated for 24 hr. The infected cells were then trypsinized and split into normal growth medium (control) or a 1:8 mixture of CM and whole growth medium. The cells were harvested 18 hr later and luciferase activity was determined. The values represent the means of two infections in a representative experiment. Fold induction was calculated by dividing the average luciferase activity in cytokine-rich medium by the average activity in control medium.

achieved *in vivo*, mice were injected intravenously with 5×10^8 to 5×10^9 plaque-forming units (pfu) of various recombinant adenoviruses. Three days later, the mice were challenged with saline, LPS, or turpentine, and 18–24 hr later luciferase activity was measured in liver, spleen, heart, lung, and kidney. At high viral inocula ($>2 \times 10^9$ pfu), we were unable to demonstrate consistent induction from the C3 promoter. Since recombinant adenoviruses can induce hepatitis (17), we measured serum aminotransferase activities in a sample of these mice and found significant elevations [alanine aminotransferase, 465 ± 191 units/liter; aspartate aminotransferase, 1310 ± 820 units/liter (mean ± SD, $n = 14$)] above the levels found in saline-injected control mice [alanine aminotransferase, 48 ± 6; aspartate aminotransferase, 86 ± 16 ($n = 35$)]. When lower viral inocula (5×10^8–1×10^9 pfu) were used, normal serum aminotransferase levels were observed and unequivocal induction of the C3 and SAA3 promoter constructs was demonstrable.

Time Course of Inducible Luciferase Expression. Fig. 1 shows the time course of hepatic luciferase expression from Ad.SAA3-Luc (*Upper*) and Ad.C3-Luc (*Lower*) after intraperitoneal LPS challenge. Luciferase expression from both constructs increased rapidly, remained at high levels (≈170-fold above baseline) for several hours, and then gradually declined. SAP levels, in contrast, increased slowly over the same time period (Fig. 1 *Upper*), as previously reported (18). To exclude the possibility that the decrease in luciferase activity shown in Fig. 1 resulted from the loss of the virally transferred gene during the 24-hr observation period, a group of Ad.C3-Luc-infected mice was also injected with LPS at the end of the 24-hr period (i.e., at 4 days after viral infection); 3 hr later, the logarithm of the hepatic luciferase activity in these mice was 4.1 ± 0.4 (mean ± SD, $n = 5$), comparable to that seen at the 3-hr post-LPS time point 1 day earlier.

Dose-Dependent Expression from the SAA3 Promoter *in Vivo*. We next measured luciferase expression from the SAA3 promoter in response to increasing doses of LPS. Mice were injected with Ad.SAA3-Luc (7.5×10^8 pfu) and challenged 3 days later with saline or with 25, 75, or 225 μg of LPS. As shown in Fig. 2, there was a positive correlation between the LPS dose and the level of induction. Relative to saline-treated animals, mean luciferase activity was 10-, 170-, and 220-fold higher in mice receiving 25, 75, and 225 μg of LPS, respectively ($P <$ 0.002 for trend).

Comparison of the SAA3 and C3 Promoters *in Vivo*. The SAA3 and C3 promoters were both inducible by inflammatory stimuli *in vivo*. When challenged with LPS 18–20 hr earlier, mice infected with Ad.SAA3-Luc had mean liver luciferase activities that were 59-fold those found in saline-challenged controls. When challenged with turpentine, however, luciferase expression from Ad.SAA3-Luc was only 3-fold above baseline. In contrast, in mice infected with Ad.C3-Luc, liver

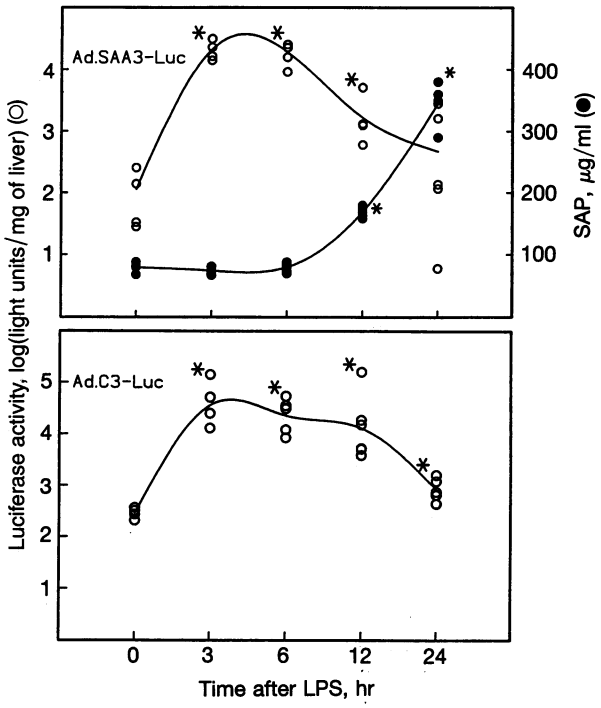

FIG. 1. Time course of liver luciferase activity and SAP after injection of LPS. Each animal was infected with 10^9 pfu of Ad.SAA3-Luc (*Upper*) or Ad.C3-Luc (*Lower*) 3 days previously. After injection of LPS (100 μg, i.p.), samples of serum and liver were obtained at the indicated times ("0" time = 5–10 min). ○, Luciferase activity; ●, SAP concentration. Each symbol represents one mouse ($n = 4$–6 mice per group). *, Significantly different from 0 time point ($P < 0.05$). Note logarithmic scale on left ordinate.

luciferase activities increased an average of 29-fold in response to 100 μg of LPS (range, 24- to 34-fold, two experiments) and 12-fold in response to turpentine (range, 6- to 19-fold, three experiments). Paradoxically, expression from the CMV promoter, which had been stimulated by cytokine-rich CM *in vitro*, decreased by a factor of 3 in response to LPS *in vivo*. SAP levels, measured in the same mice, confirmed that an acute-phase response occurred in mice with either turpentine or LPS challenge (Fig. 3).

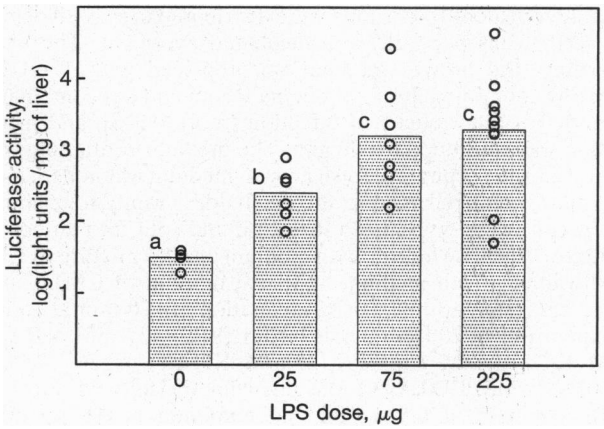

FIG. 2. Response of the SAA3 promoter to graded doses of LPS. Mice infected with Ad.SAA3-Luc were challenged intraperitoneally 3 days later with saline or the indicated doses of LPS. Hepatic luciferase activity was measured after the animals were observed for 18 hr. Each open circle represents one mouse. Heights of bars indicate means; groups significantly different from others ($P \leq 0.05$) are indicated by letters (a, b, c) above bars.

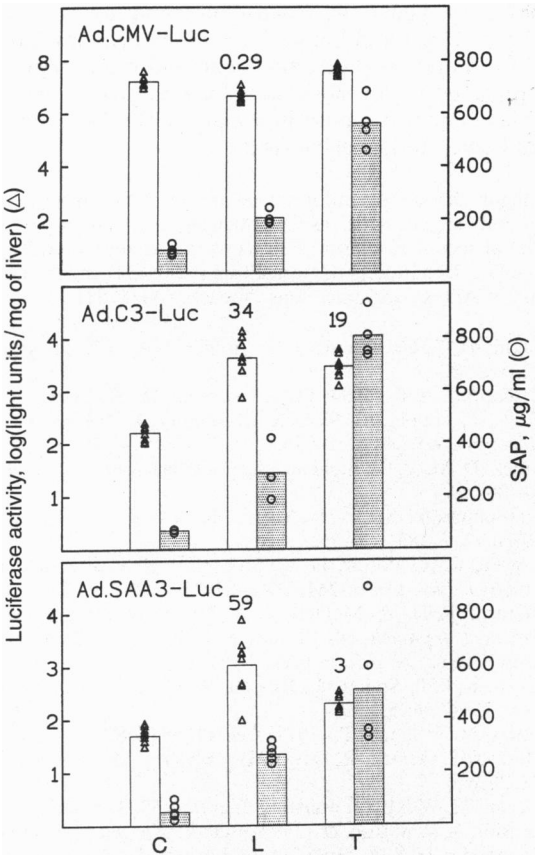

FIG. 3. Responses of adenovirus-infected mice injected with saline (control, C), 200 μg of LPS (L), or turpentine (T). Each open symbol represents results from a single mouse; heights of bars indicate means; n = 7–9 mice per group. Open bars and triangles: luciferase activity in liver lysates. Note logarithmic scale on left ordinate. The numbers above groups indicate fold increases that were significantly different from C ($P < 0.05$). Stippled bars and open circles: SAP levels in serum obtained from four or five mice in each group. Note linear scale on right ordinate. Each L and T group was different from C in each experiment ($P < 0.05$). (*Top*) Ad.CMV-Luc. (*Middle*) Ad.C3-Luc. (*Bottom*) Ad.SAA3-Luc. Mice in each group had been infected with 10^9 pfu 3 days before challenge.

To exclude the possibility that the Ad.SAA3-Luc response to turpentine was greater at earlier time points, we challenged Ad.SAA3-Luc-infected mice with saline, 100 μg of LPS, or turpentine and measured liver luciferase activity 6 hr later. When compared with the saline-treated animals, mice that received LPS averaged 800-fold greater activity, while those that received turpentine showed no elevation (data not shown). In another control experiment, no luciferase activity above background was detectable in the livers of uninfected mice or mice infected with 2×10^9 pfu of Ad.No-Luc (promoterless luciferase) and then challenged with LPS or turpentine, despite vigorous increases in SAP (data not shown).

Although adenovirus vectors principally target the liver, expression of recombinant proteins delivered by these vectors has been demonstrated in other tissues (10). We determined luciferase activities in homogenates of spleen, kidney, heart, and lung from mice that had been injected with the different adenoviral constructs (data not shown). As expected, we found that luciferase expression from the constitutive CMV early promoter occurred in each of these tissues. In contrast, no activity was detected from the SAA3 promoter in any tissue other than liver. Expression from the C3 promoter was detected in liver and lung, but not in kidney, heart, or spleen. In lung, expression from the C3 promoter was low (115 ± 73 light units/mg of tissue) but inducible by turpentine and LPS (4-fold mean increase, three experiments, each significant at $P < 0.05$). The time course in lung paralleled that for liver shown in Fig. 1. Expression from the CMV promoter in nonhepatic tissues was not altered by inflammatory stimuli.

Serum IL-6 Levels. A striking feature of the *in vivo* experiments was the variability in luciferase expression found among the mice that received LPS. To test the hypothesis that this variability resulted from heterogeneity in the host response to LPS, we measured the concentration of IL-6 in serum obtained (immediately prior to euthanasia) from animals injected with Ad.SAA3-Luc. There was a positive correlation between individual serum IL-6 levels and hepatic luciferase expression (Fig. 4; $P < 0.001$ for trend).

DISCUSSION

The acute-phase response is a highly conserved, tightly controlled component of the host response to inflammatory stimuli. Numerous clinical data suggest that blood levels of certain APPs (e.g., C-reactive protein and SAP protein) usually increase in direct relationship to the severity of insults such as trauma, thermal injury, bacterial infection, or inflammatory diseases (19). Use of promoters for APP genes to control the production of recombinant antiinflammatory proteins *in vivo* might therefore allow regulated synthesis of these proteins in response to a host's own inflammatory mediators. Although it is impossible to predict the impact of this approach without further experimentation, it might limit the severity of the inflammatory response without interfering with its beneficial roles in host defense and immunity. Potential antiinflammatory proteins include IL-1 receptor antagonist, soluble tumor necrosis factor receptors, IL-10, and endotoxin-neutralizing proteins such as bactericidal permeability-increasing protein.

There are two general classes of APPs, those produced in response to IL-1 or similar cytokines (type 1) and those produced solely (or largely) in response to IL-6 (type 2) (20). The two type 1 APP promoters used here had been studied *in vitro* by others (5, 15), who defined the critical boundaries of the promoters and showed cytokine-inducible expression with promoter–chloramphenicol acetyltransferase plasmid constructs. In addition, the DNA sequences of the promoters contain potential binding sites for cytokine-induced transcription factors (5, 15). Based on this information, we chose these promoters to attempt cytokine-regulated expression of recombinant proteins *in vivo*. In general, our *in vivo* results with these

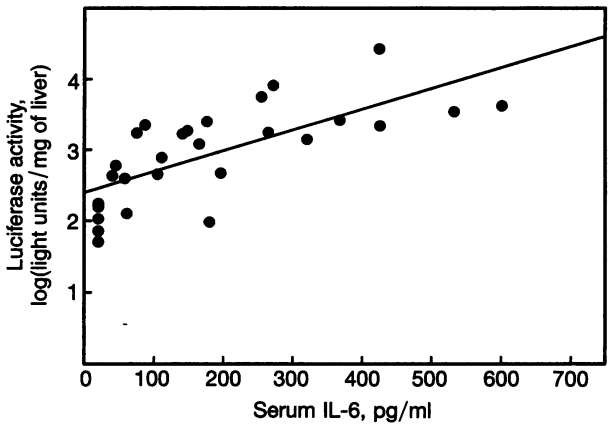

FIG. 4. Relationship between liver luciferase activity and serum IL-6 concentration. Each mouse (●; n = 28) had been injected with Ad.SAA3-Luc 4 days previously and had received an LPS injection 18–20 hr before liver and serum samples were obtained. Different animals received different LPS doses (see Figs. 2 and 3). Coefficients of correlation (r) and determination (r^2) for the line shown were 0.71 and 0.50, respectively.

APP promoters were accurately anticipated by the observations made in transfected Hep G2 cells.

Both APP promoters were highly inducible by inflammatory stimuli, exhibiting average levels of induction greatly exceeding those previously reported for other promoters *in vivo*. Two notable differences were observed in their patterns of response. (*i*) Inducible expression from the C3 promoter occurred in both liver and lung, whereas SAA3-controlled expression was found only in liver. (*ii*) The C3 promoter responded to both turpentine and LPS, whereas the SAA3 promoter was much more responsive to LPS than to turpentine. These results suggest that the behavior of different APP promoters may be used to achieve host-regulated expression of recombinant proteins in different tissues, to different degrees, and in response to different inflammatory stimuli.

Previous studies had shown that transcription from the SAA3 promoter could be induced *in vivo* by intraperitoneal LPS but not by silver nitrate (21) or casein (22), two other stimuli for eliciting acute-phase responses in animals. The modest response of Ad.SAA3-Luc to turpentine, when compared with its response to LPS, is consistent with these results and suggests that the small (306-bp) region of the SAA3 promoter used in these studies probably contains elements that influence stimulus-specific transcription.

A striking feature of all of our *in vivo* studies was the variability in the responses of individual animals to the same dose of LPS. Since blood IL-6 levels have been reported to correlate with the intensity of the inflammatory response (23), the correlation between serum IL-6 and hepatic luciferase activity (Fig. 4) suggests that much of the observed heterogeneity in luciferase expression in these animals may be attributed to variability in the inflammatory response to LPS. These data are also consistent with the conclusion that the observed increases in luciferase expression were triggered by circulating cytokines, rather than directly by the inciting agents themselves. Our results do not exclude this alternative explanation, however.

In the time-course experiment shown in Fig. 1 (*Upper*), luciferase expression began soon after LPS injection, plateaued (at about 180-fold above baseline) for several hours, and then waned. This time course is very similar to that reported for LPS-induced transcription of the endogenous murine SAA3 gene (24), suggesting that endogenous and adenovirus-mediated expression from the SAA3 promoter occur with similar rapidity. The descending arm of this curve is influenced, at least in part, by the short half-life of the firefly luciferase enzyme (25); more stable reporters may demonstrate sustained expression *in vivo*.

In contrast to the good *in vitro–in vivo* correlation seen with the APP promoters, we unexpectedly observed a difference in the performance of the CMV promoter when it was studied in cultured cells and in mice. This promoter was inducible by cytokine-rich medium *in vitro*, but expression decreased *in vivo* after animals were challenged with LPS. In contrast, administration of dexamethasone was recently reported to enhance expression from this promoter *in vivo* (26). These findings may be relevant to efforts to use the CMV promoter to deliver therapeutic proteins during inflammation (3, 27, 28).

Previous studies have shown that promoters can remain responsive to appropriate exogenous stimuli when they are transferred into cultured cells by adenovirus vectors (29, 30). An adenovirus vector containing a thyroid hormone-responsive enhancer was recently reported to mediate thyroid hormone-inducible recombinant protein expression *in vivo* (31). Our findings extend these results to two APP promoters and suggest that adenoviral vectors may be useful for delivering diverse inducible promoter constructs *in vivo*.

Our experiments have demonstrated that recombinant protein expression can be regulated *in vivo* by an animal's own inflammatory mediators. Gene delivery vectors that utilize APP promoters (or promoters for other cytokine-inducible genes) to control the production of antiinflammatory proteins could prove useful for modulating the intensity or duration of the inflammatory response in a manner that is regulated by natural homeostatic mechanisms.

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