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Serum Amyloid A-Luciferase Transgenic Mice: Response to Sepsis, Acute Arthritis, and Contact Hypersensitivity and the Effects of Proteasome Inhibition

Ning Zhang,[1] Muhammad H. Ahsan, Anthony F. Purchio, and David B. West

Acute phase serum amyloid A proteins (A-SAAs) are multifunctional apolipoproteins produced in large amounts during the acute phase of an inflammation and also during the development of chronic inflammatory diseases. In this study we present a *Saa1-luc* transgenic mouse model in which *SAA1* gene expression can be monitored by measuring luciferase activity using a noninvasive imaging system. When challenged with LPS, TNF-α, or IL-1β, in vivo imaging of *Saa1-luc* mice showed a 1000- to 3000-fold induction of luciferase activity in the hepatic region that peaked 4–7 h after treatment. The induction of liver luciferase expression was consistent with an increase in *SAA1* mRNA in the liver and a dramatic elevation of the serum SAA1 concentration. Ex vivo analyses revealed luciferase induction in many tissues, ranging from several-fold (brain) to >5000-fold (liver) after LPS or TNF-α treatment. Pretreatment of mice with the proteasome inhibitor bortezomib significantly suppressed LPS-induced *SAA1* expression. These results suggested that proteasome inhibition, perhaps through the NF-κB signaling pathway, may regulate *SAA1* expression. During the development of acute arthritis triggered by intra-articular administration of zymosan, *SAA1* expression was induced both locally at the knee joint and systemically in the liver, and the induction was significantly suppressed by bortezomib. Induction of *SAA1* expression was also demonstrated during contact hypersensitivity induced by topical application of oxazolone. These results suggest that both local and systemic induction of A-SAA occur during inflammation and may contribute to the pathogenesis of chronic inflammatory diseases associated with amyloid deposition. ***The Journal of Immunology,*** **2005, 174: 8125–8134.**

Acute phase serum amyloid A proteins (A-SAAs)[2] are multifunctional apolipoproteins that are secreted in the acute phase of inflammation. These proteins are found in the high density lipoprotein fraction of the serum and are involved in transporting cholesterol to liver for secretion into the bile, induction of extracellular matrix-degrading enzymes, and chemotactic recruitment of inflammatory cells to the site of inflammation. A-SAAs are involved in the pathogenesis of several chronic inflammatory diseases, such as amyloidosis, atherosclerosis, and rheumatoid arthritis (1, 2).

In the mouse, three acute phase *SAA* isoforms have been reported. Murine *SAA1* and *SAA2* isoforms are expressed and induced predominantly in the liver, whereas the SAA3 isoform is induced in multiple tissues during inflammation (3, 4). The *SAA1* and *SAA2* genes in the BALB/C strain are 72% identical over the first 500 bp upstream of their transcription start sites (5). Both genes are similarly regulated by the proinflammatory cytokines IL-1, IL-6, and TNF-α in hepatic cells (6). The regulation of *SAA* genes involves a number of transcription factors, such as NF-κB, C/EBPs, AP-2, Yin Yang 1, sequence-binding factor, and specificity protein 1 (4, 7–11). Under acute inflammatory conditions triggered by LPS, both SAA1 and SAA2 are induced to a similar extent, causing up to a 1000-fold increase in the serum A-SAA concentration (3). The induction of *SAA1* and *SAA2* during inflammation is largely triggered by elevated levels of the proinflammatory cytokines, IL-1, IL-6, and TNF-α, in the circulation (12–14).

Synthesis of A-SAAs in nonhepatic tissues has been documented. A-SAAs have been found in the synovial fluid recovered from inflamed joints of rheumatoid arthritis patients (15), and it is synthesized locally in the synovial membrane (16) by fibroblasts (17) and chondrocytes (18) as a result of the presence of high levels of proinflammatory cytokines, such as IL-1 and IL-6 in the synovial fluid of these patients (19). It is believed that locally synthesized SAA by synovial cells in the inflamed joints acts as an autocrine inducer of matrix metalloproteinase-1 (collagenase), the only enzyme that degrades interstitial collagens I, II, and III and causes extensive joint erosion (17, 18, 20). In addition, induction of SAA by proinflammatory cytokines has been reported in many other cell types, including macrophages, endothelial cells, smooth muscle cells, and adipocytes of coronary and carotid arteries in human atherosclerotic lesions (21); intestinal epithelial cells (22); and the brain of patients with Alzheimer's disease (23). The extrahepatic expression of *SAAs* suggests their involvement in the pathogenesis of these chronic diseases.

A-SAA and C-reactive protein are currently the most sensitive indicators for assessing inflammatory activity (24). Development of convenient in vivo assays for A-SAA will facilitate its use in the preclinical evaluation of anti-inflammatory drugs. For this purpose, we have established a *Saa1-luc* transgenic mouse model using the murine *SAA1* promoter to direct the expression of a luciferase reporter. In conjunction with a highly sensitive IVIS imaging system (Xenogen), we have applied this model to noninvasively monitor SAA1 expression under various inflammatory conditions, such as sepsis, acute arthritis, and chemically induced hypersensitive reaction of the skin. The results presented in this report suggest that the *Saa1-luc* transgenic mouse offers a convenient and sensitive approach for examining in vivo SAA1 expression under various inflammatory diseases conditions.

Xenogen, Alameda, CA 94501

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[1] Address correspondence and reprint requests to Dr. Ning Zhang, Xenogen, 860 Atlantic Avenue, Alameda, CA 94501. E-mail address: ning.zhang@xenogen.com

[2] Abbreviations used in this paper: A-SAA, acute phase serum amyloid A protein; CHS, contact hypersensitivity.

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Materials and Methods

*Construction of p*Saa1-luc *vector and generation of* Saa1-luc *transgenic mice*

A mouse *SAA1* bacterial artificial chromosome clone was obtained through PCR screening of a mouse C57BL/6J genomic DNA library (Research Genetics). A promoter fragment of 7.7 kb was cloned into the pGL3Basic vector (Promega) upstream of the firefly luciferase gene sequence, and the construct was designated p*Saa1-luc.* The *Saa1-luc* transgene cassette was released from the vector through double digestions with *Not*I and *Sal*I and was used to generate transgenic mice in the BALB/C background strain with standard pronuclear microinjection techniques. The transgenic model described in this paper is named BALB/C-Tg (*Saa1-luc*)Xen and is abbreviated *Saa1-luc* throughout the text.

Reagents

We purchased bacterial LPS (from *Salmonella abortus equi*) and zymosan A (a cell wall preparation from *Saccharomyces cerevisiae*) from Sigma-Aldrich, rTNF-α and IL-1β from R&D Systems, and bortezomib (velcade, PS-341) from Millennium Pharmaceuticals.

In vivo imaging of luciferase activity

In vivo imaging was performed using an IVIS imaging system (Xenogen) as previously described (25). *Saa1-luc* transgenic mice were anesthetized with isoflurane and injected i.p. with 150 mg/kg luciferin (Biosynth). Ten minutes after the luciferin injection, mice were imaged for 1–60 s. Photons emitted from specific regions were quantified using LivingImage software (Xenogen). In vivo luciferase activity is expressed as photons per second.

Induction of SAA1 promoter-driven luciferase expression in transgenic animals

Induction of *SAA1* promoter-driven luciferase expression was followed after i.p. injection of LPS (1 mg/kg), IL-1β (1 μg/mouse), or TNF-α (2 μg/mouse) into *Saa1-luc* transgenic mice, 3–6 mo of age. Control mice were injected with saline. At selected time points after the treatment, mice were i.p. injected with luciferin and were imaged 10 min later with the IVIS imaging system described above. To test the effect of bortezomib on LPS-triggered luciferase expression, mice were pretreated with bortezomib (1 mg/kg i.v.) 1 h before LPS injection, and the luciferase signal was monitored through imaging.

Tissue luciferase activity

Selected mouse organs were removed, homogenized in 3 vol of PBS containing a protease inhibitor mixture (Roche), and lysed with passive lysis buffer (Promega). After centrifugation at 14,000 rpm for 10 min at 4°C, the supernatant was collected. Luciferase activity was assayed using the Luciferase Assay System (Promega) and a TD 20/20 luminometer (Turner Design). Protein concentration was estimated with Bradford reagent (Sigma-Aldrich).

Acute arthritis model by intra-articular administration of zymosan

Zymosan A was suspended in sterile saline containing 5% glucose at a concentration of 30 mg/ml. *Saa1-luc* mice, 3–6 mo of age, were anesthetized with isoflurane, the hind legs of the mice were shaved, and the skin was sterilized with 70% ethanol. The right knee tendon was exposed, and 10 μl of zymosan A suspension was injected intra-articularly through the tendon with a 25-gauge needle. The left rear knees of the same mice were intra-articularly injected with 5% glucose in saline (sham control). Mice were imaged at selected time points after the injection. To test the effect of bortezomib on zymosan-triggered luciferase expression, mice were pretreated with bortezomib (1 mg/kg i.v.) 1 h before zymosan administration, and the luciferase signal was monitored through imaging.

Induction of contact hypersensitivity (CHS) reactions

Male *Saa1-luc* mice, 3–6 mo of age, were shaved in the thorax area and treated topically with 50 μl of 2% oxazolone solution (in acetone/olive oil (4/1, v/v)). Control mice were treated with vehicle (acetone/olive oil (4/1)) alone. Bioluminescent images were collected at various time points after treatment.

Measurement of SAA1 in serum and tissues by ELISA

A mouse SAA1 immunoassay kit (BioSource International) was used to measure SAA1 level in the serum. Both male and female *Saa1-luc* mice (n = 6), 3–6 mo of age, were injected with LPS (1 mg/kg i.p.) or TNF-α (2 μg/mouse i.p). Blood samples were collected 4 h after the treatment. Before the ELISA, serum samples from LPS- or TNF-α-treated mice were diluted 1/1000 in sample diluent. Control serum samples were diluted 1/10 in sample diluent. Samples were measured for the SAA1 concentration following the manufacturer's protocol.

We also measured local SAA1 production in the zymosan-injected knee joint or the oxazolone-treated skin area. The knee joint or skin tissue was harvested and ground into fine power while frozen with liquid nitrogen, followed by brief sonication in 3 vol of PBS containing a protease inhibitor mixture (Roche). The homogenate was additionally lysed with passive lysis buffer (100 μl of homogenate, 300 μl of PBS, and 100 μl of 5× passive lysis buffer (Promega)). After centrifugation at 14,000 rpm for 10 min at 4°C, the supernatant was collected for measurement of SAA1 concentration, luciferase activity, and total protein, as described above. No dilution was needed for the ELISA.

Northern blot analysis

Total RNA was isolated from mouse tissue using RNAwiz (Ambion) and further purified using an RNeasy kit (Qiagen). A total of 5 μg of RNA was analyzed by Northern blot using a NorthernMax system (Ambion). A 25-nt SAA1-specific probe (5′-CCTTGGAAAGCCTCGTGAACAAATG-3′) was used for hybridization to the RNA filter at 37°C in ULTRAhyb-Oligo hybridization buffer (Ambion). After hybridization, the signal was detected using a BrightStar BioDetect kit (Ambion).

Statistics

Nonparametric tests for significance were used to test whether changes in luciferase signal from baseline were significantly greater than zero (sign test) and whether the changes from baseline were significantly different between treatment groups (Mann-Whitney U test). Values are presented as the mean ± 1 SE in the graphs and text unless otherwise noted. For some statistical tests, genders were combined to increase sample number in each group. All significance levels are two-sided.

Results

Induction of SAA1 promoter-driven luciferase expression by LPS and TNF-α *in* Saa1-luc *transgenic mice*

We generated *Saa1-luc* transgenic founders whose progeny were screened for luciferase expression in response to LPS as described in *Materials and Methods*. All founder lines showed robust luciferase induction in the liver after LPS treatment. One founding line was selected for the studies described in this report. Fig. 1 shows the results of treatment with LPS, TNF-α, and IL-1β. Luciferase expression was detected by bioluminescent imaging. LPS treatment caused a robust induction of luciferase signal in the hepatic region of the transgenic mice. Induction was detectable 2 h after LPS treatment and reached a peak at 4–7 h. The signal was reduced sharply at 24 h, and by 48 h the signal had returned to the baseline level. Control mice that were injected with saline did not show any induction (results not shown).

Luciferase signal from the liver region was quantified using LivingImage software to produce the data shown in Fig. 2 (*upper panel*). Male mice had significantly higher mean luciferase signals than female mice at baseline (mean ± SE, 142.7 ± 62.6 vs 25.8 ± 24.9 million photons/s, respectively; $p < 0.0001$). After LPS treatment, both males and females showed significant induction of luciferase signal at all time points compared with the baseline level. In relation to the baseline, the fold increase in luciferase signal was similar in males and females (Fig. 2, *right top panel*). Male mice showed a peak of luciferase induction by 4 h, whereas female mice had a peak of induction at 7 h. At the peak, the luciferase signal was induced ~2400-fold in both sexes (difference between sexes, $p = 0.57$). Neither sex exhibited induction after saline injection (results not shown). In another experiment, we found that there was no difference by age for luciferase induction after LPS injection among mice aged 2–7 mo (data not shown).

Robust luciferase induction was also observed in the hepatic region of both sexes after i.p. injection of either TNF-α (Fig. 1,

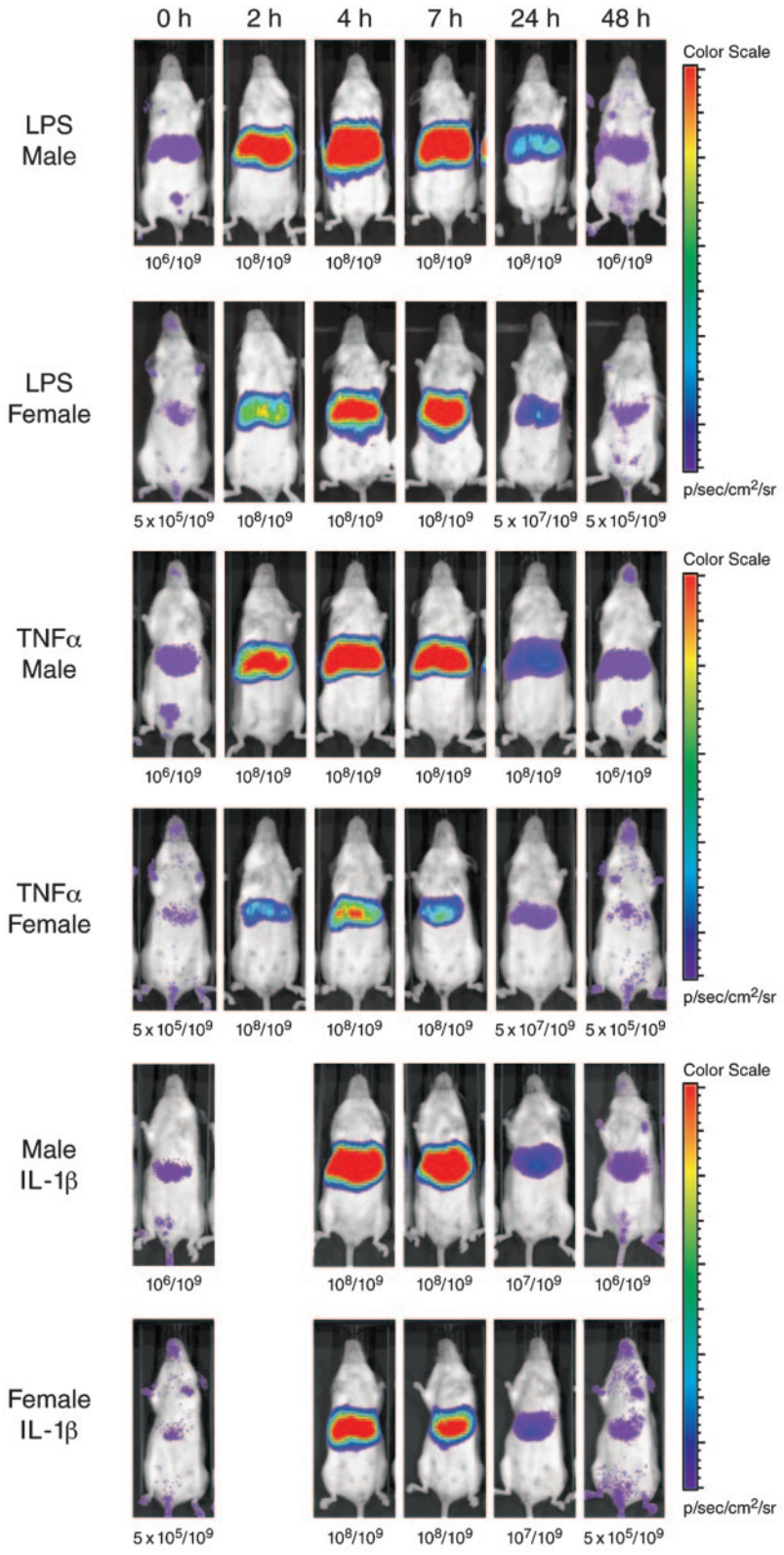

FIGURE 1. In vivo IVIS imaging (Xenogen) of anesthetized 3- to 6-mo-old *Saa1-luc* transgenic male and female mice after i.p. injection of 150 mg/kg luciferin. The mice were treated with LPS (1 mg/kg; *top panel*), TNF-α (2 μg/mouse; *middle panel*), or IL-1β (1 μg/mouse; *bottom panel*) and imaged at 0, 2, 4, 7, 24, and 48 h after treatment. Representative mice from each treatment group are shown. The color overlay on the image represents the photons per second emitted from the animal in accord with the pseudocolor scale shown next to the images. Red represents the highest number of photons per second, whereas blue represents the lowest number of photons per second. After all three treatments, luciferase activity was clearly induced in the region of the liver.

middle panel) or IL-1β (Fig. 1, *bottom panel*). The *middle* and *lower panels* of Fig. 2 display the corresponding kinetics of luciferase induction. The luciferase signal was readily detectable 2 h after TNF-α injection, reached a peak at 4 h, started to decline at 7 h, and by 48 h was only marginally above the baseline. The fold increases in luciferase signal for the sexes combined (n = 6/group) were significantly greater than zero at all time points (p = 0.03; Fig. 2). The kinetics were similar for hepatic luciferase induction by IL-1β. The fold increase in luciferase signal after treatment with IL-1β showed no statistically significant difference between sexes. When data from both sexes were combined, there was a significant positive increase induced by IL-1β 4, 7, 24, and 48 h after treatment (p = 0.03; Fig. 2).

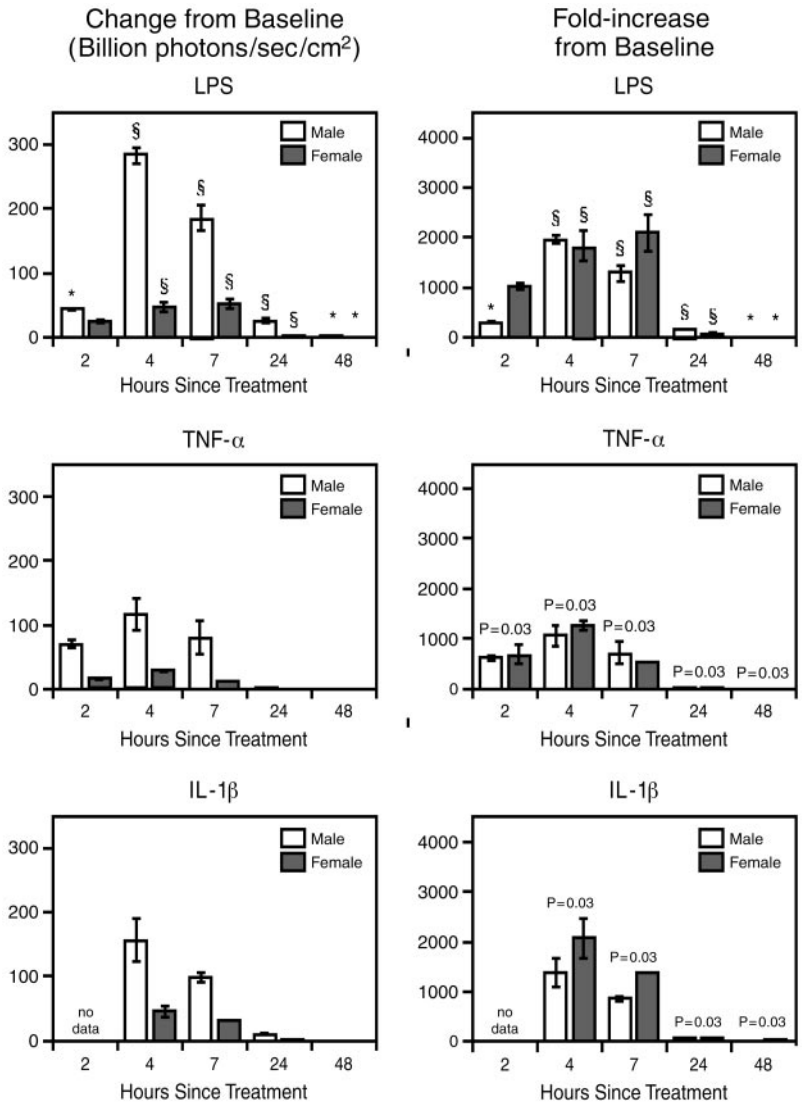

FIGURE 2. Change in luciferase activity over time, as expressed by the difference between baseline photons per second and subsequent measurements (*left panel*), and as multiples of the baseline activity (*right panel*). Values are the mean increase ± SE or the fold increase ± SE. Nonparametric significance levels from the sign test: ∗, $0.01 < p \leq 0.05$; §, $p < 0.0001$. In the *top panels*, 18 male and 14 female *Saa1-luc* transgenic mice were measured at baseline and 4, 7, and 24 h after LPS injection. Two subsets of mice treated with LPS received supplementary imaging at 2 h (seven males and three females) and 48 h (six males and six females) after LPS treatment. In the *middle* and *lower panels*, *Saa1-luc* transgenic mice (three males and three females) were treated with TNF-α (2 μg/mouse i.p) or IL-1β (1 μg/mouse i.p.) and imaged at 2 h (TNF-α only) and 4, 7, 24, and 48 h after treatment. When the sexes were combined for statistical analysis, there were significant fold increases relative to baseline at all time points after both TNF-α and IL-1β treatments ($p = 0.03$).

Induction of SAA1 promoter-driven luciferase expression by LPS and TNF-α corresponded with the induction of serum SAA1

We analyzed the serum level of SAA1 after LPS or TNF-α treatment. Mice were bled 4 h after treatment, and the SAA1 serum concentration was tested by ELISA. The concentrations of SAA1 in untreated control mice (mean ± SE) were 0.6 ± 0.1 and 0.7 ± 0.1 μg/ml in males and females, respectively. The SAA1 level was raised to an average of 1793 ± 117 and 1318 ± 315 μg/ml in male LPS- and TNF-α-treated mice, respectively, representing 2846- and 2092-fold inductions. In female mice, the SAA1 levels were raised to averages of 1435 ± 148 and 1232 ± 249 μg/ml after LPS and TNF-α treatment, respectively, representing 2119- and 1820-fold inductions for LPS and TNF-α, respectively. The mean serum SAA1 levels in LPS- and TNF-α-treated mice were all significantly greater than the control values ($p = 0.004$), and the fold increases in serum SAA1 induction measured by ELISA agreed well with those obtained by bioluminescent imaging.

SAA1 promoter-driven luciferase expression is induced in multiple tissues after LPS and TNF-α treatment

We analyzed luciferase activity in selected organs in *Saa1-luc* mice 4 h after i.p. injection of LPS, TNF-α, or saline. Ex vivo luciferase activity was detected in the liver and in all other dissected organs of LPS-, TNF-α-, and saline-injected mice (Table I). However, induction of luciferase activity was significantly greater in the LPS- and TNF-α-treated than in the saline-injected mice for liver, lungs, kidneys, brain, and heart. In the intestine, LPS produced significant induction of luciferase in both male and female tissues, whereas TNF-α produced significant induction in male, but not female, intestinal samples. In the spleen, induction was significant after both LPS and TNF-α treatments in male, but not female, mice. As calculated from the mean of the saline-injected mice, LPS treatment in male and female mice caused luciferase induction of 4508- and 5762-fold, respectively, in the liver, 489- and 208-fold in the lungs, 68- and 30-fold in the small intestines, 71- and 75-fold in the kidneys, 1.6- and 1.5-fold in the brain, 68- and 21-fold in the heart, and 2.1- and 1.6-fold in the spleen. The corresponding values for TNF-α treatment in male and female mice were inductions of 3016- and 2288-fold, respectively, in the liver, 67- and 18-fold in the lungs, 329- and 4-fold in the small intestines, 19- and 56-fold in the kidneys, 0.7- and 0.7-fold in the brain, 53- and 12-fold in the heart, and 1.5- and 1.7-fold in the spleen.

Consistent with the in vivo results at 4 h in Fig. 1, the ex vivo analyses described above of luciferase induction in selected organs showed that males had significantly greater induction than females

Table I. *Ex vivo luciferase expression*[a]

	Mean ± SE						Significance[b]			
	LPS		TNF-α		Saline		LPS vs Saline		TNF-α vs Saline	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Liver	$10.3 \pm 1.6 \times 10^6$	$0.56 \pm 0.14 \times 10^6$	$6.9 \pm 0.5 \times 10^6$	$0.22 \pm 0.11 \times 10^6$	2,287 ± 885	98 ± 12	0.05	0.05	0.05	0.05
Lung	27,164 ± 1,325	21,608 ± 910	3,734 ± 751	1,853 ± 331	55 ± 13	104 ± 40	0.05	0.05	0.05	0.05
Intestine	3,144 ± 605	1,291 ± 164	15,282 ± 9,394	156 ± 67	46 ± 10	43 ± 12	0.05	0.05	0.05	0.12
Spleen	2,653 ± 697	1,769 ± 97	1,878 ± 204	1,894 ± 267	1,251 ± 164	1,096 ± 263	0.05	0.12	0.05	0.12
Kidney	2,072 ± 576	2,560 ± 717	548 ± 211	1,908 ± 1076	29 ± 4	34 ± 6	0.05	0.05	0.05	0.05
Brain	679 ± 53	629 ± 29	305 ± 25	277 ± 38	418 ± 32	410 ± 6	0.05	0.05	0.05	0.05
Heart	1,026 ± 433	271 ± 28	795 ± 331	156 ± 63	15 ± 2	13 ± 2	0.05	0.05	0.05	0.05

[a] Ex vivo luciferase expression (units per milligram of protein) in selected organs from *Saa1-luc* mice (six mice (three male, three female) per treatment) 4 h post-i.p. injection with LPS, TNF-α, or saline.
[b] Nonparametric Mann-Whitney test.

in the liver, lungs, intestines, and heart ($p = 0.05$) after either LPS or TNF-α treatment. Despite differences in the amount of induction, male and female LPS-treated mice showed a similar pattern of increased luciferase expression across organs. Liver demonstrated by far the greatest increase in luciferase expression in both sexes, whereas brain and spleen demonstrated the smallest increases. In male and female LPS-treated and TNF-α-treated mice, the luciferase activity in all other tissues was significantly less than that in the liver (Table I).

Induction of SAA1 promoter-driven luciferase expression by LPS is inhibited by bortezomib

The presence of NF-κB-responsive elements in the *SAA1* promoter suggests that the NF-κB signaling pathway is involved in the regulation of *SAA1* expression. We studied the effect of bortezomib on *SAA1* promoter-driven luciferase induction by LPS. Bortezomib is a proteasome inhibitor that inhibits IκB degradation and therefore suppresses NF-κB-mediated induction of transcription. *Saa1-luc* mice were pretreated with bortezomib (1 mg/kg i.v.) 1 h before LPS treatment. Fig. 3 (*top panel*) shows that compared with *Saa1-luc* mice treated with LPS alone, the bortezomib-cotreated *Saa1-luc* mice showed less induction of luciferase signal in the hepatic region. Fig. 4*A* shows that luciferase induction was observed 2 h after LPS treatment and reached a peak at 4 h. At the peak, LPS-treated mice showed a 2888 ± 764-fold induction of hepatic luciferase signal. Mice pretreated with bortezomib showed only a 301 ± 50-fold induction at 4 h, which represented a 90% inhibition of luciferase induction, and this inhibition by bortezomib persisted through 24 h after treatment. The bortezomib inhibition was statistically significant at all time points whether measured as absolute increases in luciferase signal or fold increase from baseline (not displayed).

Suppression of LPS-induced SAA1 expression by bortezomib occurred in multiple tissues

Because *SAA1* promoter-driven luciferase induction by LPS occurred in several tissues, we investigated whether the suppression by bortezomib of luciferase occurred in all these tissues as well. *Saa1-luc* mice were pretreated with bortezomib (1 mg/kg i.v.) 1 h before LPS treatment. Positive control mice were treated with LPS alone, and negative control mice received saline. Selected organs were removed 4 h after LPS treatment and processed for luciferase activity; the data are presented in Table II. Consistent with the findings shown in Table I, the luciferase signal was significantly induced in all organs of LPS-treated mice compared with that in mice treated with saline. Mice pretreated with bortezomib showed significant inhibition of luciferase induction in their liver, lungs, kidney, and brains compared with mice that received only LPS. Compared with luciferase activity in the organs of control mice, LPS treatment caused a >9000-fold induction of liver luciferase activity, which was reduced by >80% by pretreatment with bortezomib. In lungs, kidney, and heart, the induction of luciferase expression was 200- to 400-fold by LPS, which was reduced by >60% with bortezomib (Table II). Serum SAA1 levels 4 h after LPS treatment were significantly lower ($p < 0.05$) in mice pretreated with bortezomib (mean ± SE, 45 ± 7 μg/ml) than in those without pretreatment (1426 ± 46 μg/ml).

We analyzed the endogenous SAA1 mRNA expression in liver tissue, as shown in Fig. 4*B*. In liver tissue of control mice, *SAA1* mRNA expression was not detectable. An induction of *SAA1* mRNA expression was observed in the livers of LPS-treated male and female mice. Induction of *SAA1* mRNA expression by LPS was inhibited by bortezomib pretreatment. Thus, the luciferase activity correlated with the endogenous *SAA1* mRNA expression in liver tissue. We also analyzed *SAA1* mRNA expression in other nonhepatic tissues from control, LPS-treated, and bortezomib-pretreated mice. Due to the low sensitivity of the assay, we were unable to detect SAA1 message in any of the nonhepatic tissues (data not shown).

SAA1 promoter-driven luciferase expression is induced during acute arthritis, and the induction is suppressed by bortezomib

We next used the *Saa1-luc* model to study SAA1 promoter-driven luciferase expression during arthritis development. In this study, female *Saa1-luc* mice, 3–6 mo of age, were used for the induction of acute arthritis by intra-articular zymosan injection. As shown in Fig. 3 (*middle panel*), injection of zymosan into the right knee joint of *Saa1-luc* mice induced luciferase expression at the knee joint. In addition, robust induction occurred in the liver. The induction of luciferase signal in knee joints and liver was detectable at 4 h, peaked at 7 h, and declined by 24 h after injection. At 24 and 120 h, the liver signal declined almost to the baseline level, whereas the signal from the knee joints remained induced. The control left knee that was injected with vehicle showed little change in luciferase activity. Pretreatment of the mice with bortezomib (1 mg/kg i.v.) significantly reduced luciferase induction in both liver and knee joint at 4 and 7 h ($p < 0.05$). Quantification of luciferase signal at the hepatic region and knee joint is presented in Fig. 5. The zymosan-injected right knee joint showed 14-, 24-, 7-, and 6-fold inductions of luciferase signal at 4, 7, 24, and 120 h, respectively (Fig. 5*A*). Bortezomib pretreatment moderately inhibited the signal from the right knee joint by ~50% at 4 and 7 h and did not seem to affect the signal 24 and 120 h after zymosan injection (Fig. 5*A*). The control left knee showed slight induction, possibly caused by a systemic response to zymosan administration

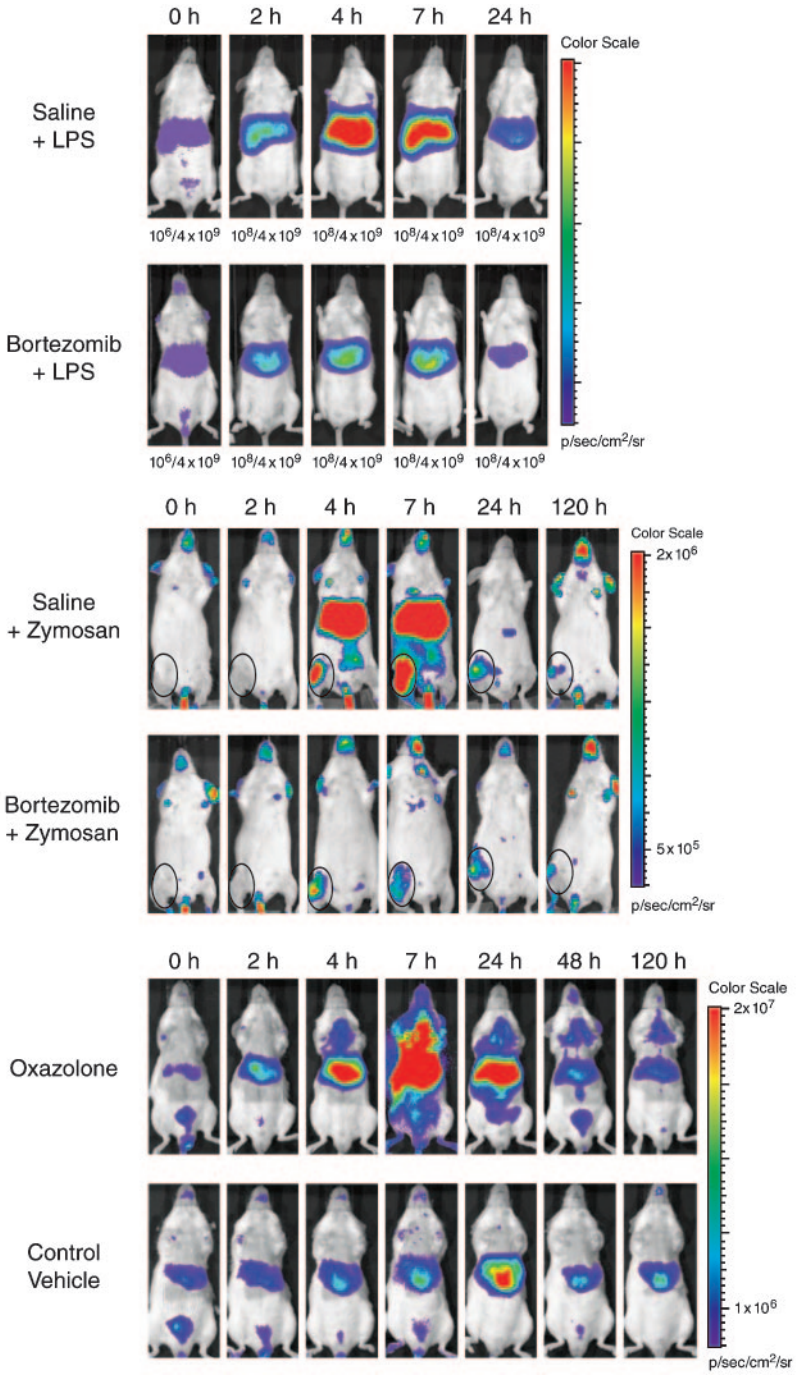

FIGURE 3. In vivo IVIS imaging of anesthetized, 3- to 6-mo-old *Saa1-luc* transgenic mice. Representative mice are shown for each group. *Top panel*, Male mice were pretreated with bortezomib (1 mg/kg i.v.) or saline 1 h before injection of LPS, (1 mg/kg i.p.). *Middle panel*, Female *Saa1-luc* transgenic mice were pretreated with bortezomib (1 mg/kg i.v.) or saline 1 h before intra-articular injection of zymosan (300 μg/knee joint) into the right rear knee joint and vehicle (5% glucose in saline) into the left rear knee joint. The zymosan-injected right knee area or the saline-injected left knee area was measured for total emission of photons. *Bottom panel*, Male *Saa1-luc* transgenic mice received oxazolone or vehicle topically to the shaved thorax area. The times shown represent hours after treatment. The color overlay on the image represents the photons per second emitted from the animal in accordance with the pseudo-color scale shown above the images. Red represents the highest number of photons per second, and blue represents the lowest number of photons per second.

(Fig. 5*B*). In the hepatic region, the luciferase signal was highly induced 4 and 7 h after zymosan injection, whereas the pretreated mice showed <2-fold induction (Fig. 5*C*). We also measured SAA1 protein production locally at the knee joint after intra-articular injection of either saline or zymosan. As shown in Fig. 5*D*, the zymosan-injected knee joint had an SAA1 level of 0.42 μg/mg total protein, which is ~12-fold higher than that in the saline-treated control knee joint.

SAA1 promoter-driven luciferase expression is induced during CHS reaction

We investigated the response of *SAA1* promoter-driven luciferase expression during CHS reaction triggered by topical application of oxazolone. As shown in Fig. 3 (*lower panel*), application of oxazolone triggered induction of luciferase expression in the treated area and hepatic region. The induction was first observed 4 h after treatment and peaked at 7 h in both the treated area and the liver. The induction was still high at 24 h. After that, the liver signal declined rapidly, whereas the signal from the treated area declined much more slowly and was still detected at 120 h. The vehicle-treated control mice showed a slight response. Quantification of the liver and oxazolone-treated skin area (Fig. 6, *A* and *B*) showed that at the treated area, induction of luciferase in oxazolone-treated mice was significantly greater than that in mice treated with vehicle alone at 4, 7, 24, and 48 h after treatment. Significantly greater induction in the hepatic region occurred in oxazolone-treated mice relative to application of the vehicle 4 and 24 h after treatment (marginally greater at 7 h, $p = 0.06$). A 264-fold induction of luciferase activity was detected at 7 h in the hepatic region of

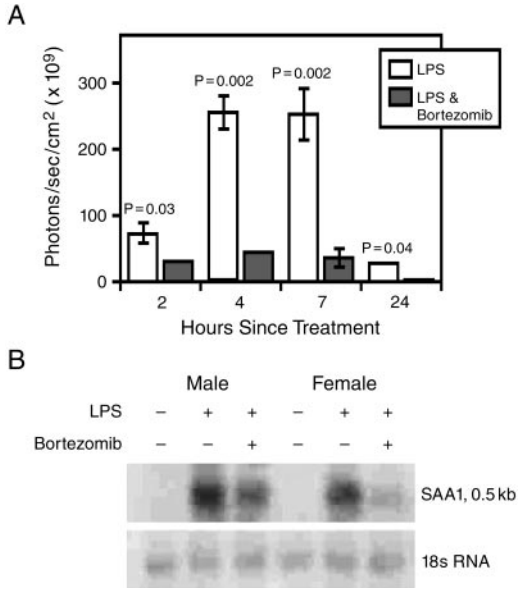

FIGURE 4. *A*, Change in liver region luciferase signal over time in male *Saa1-luc* transgenic mice pretreated with bortezomib (1 mg/kg i.v.; $n = 10$) or saline ($n = 5$) 1 h before injection of LPS (1 mg/kg i.p.). Five of the 10 bortezomib-pretreated mice were imaged at 24 h. Mean changes from baseline ± SE are presented. Nonparametric significance levels for the difference between treatment groups were determined by Mann-Whitney *U* test and are presented above the bars. *B*, Northern blot analysis of SAA1 mRNA. Induction of SAA1 mRNA expression was demonstrated in liver tissue from male and female mice treated with LPS (1 mg/kg i.p.) compared with that in control liver tissue. Pretreatment of the mice with bortezomib (1 mg/kg i.v.) inhibited LPS-induced SAA1 mRNA expression in the liver. Each RNA sample was prepared using tissues collected from three mice that were equally mixed. Equal loading was demonstrated by 18S rRNA.

oxazolone-treated mice. For the oxazolone-treated area, we detected a 39-fold induction of luciferase activity at the peak of 7 h, whereas the control vehicle-treated area showed a 2.5-fold induction. At 120 h in vehicle-treated mice, the signal from the liver and the vehicle-treated area declined to the basal level. In oxazolone-treated mice, the liver signal declined to the basal level, whereas the signal from the treated area was still 3-fold above the basal level at 120 h (Fig. 6, *A* and *B*). Induction of SAA1 protein synthesis in the oxazolone-treated skin area was confirmed by ELISA. As shown in Fig. 6*C*, oxazolone-treated skin tissue had a SAA1 level of 0.51 μg/mg total protein, which is ~19-fold higher than that in vehicle-treated control skin tissue.

Discussion

A-SAA is regarded as a sensitive indicator for assessing inflammatory activity (24). Development of a convenient in vivo assay for monitoring A-SAA production should provide a powerful tool for monitoring various inflammatory processes. We have generated a murine *Saa1-luc* transgenic mouse for monitoring *SAA1* transcriptional activity during inflammation. Using the IVIS imaging system, we demonstrated that luciferase signals in *Saa1-luc* transgenic mice were dramatically induced in the liver after treatment with LPS, IL-1β, or TNF-α. The fold induction of luciferase activity matches the fold induction of serum *SAA1* protein levels in LPS- or TNF-α-treated mice. Under other inflammatory conditions, such as arthritis and CHS reaction, luciferase signal was induced both locally in the treated area and systemically in the liver. These data indicate that *Saa1-luc* mice are useful as a sensitive and convenient model for monitoring SAA1 expression during the disease process in a broad range of inflammatory conditions.

The murine SAA1 promoter was isolated from a bacterial artificial chromosome clone of a C57BL/6J genomic DNA library. The five members of the mouse SAA family span 45 kb on chromosome 7p (2). The *SAA1* and *SAA2* genes are oppositely oriented on the chromosome. The distance between the ATG translational start codon of the *SAA1* and *SAA2* genes is 9.8 kb. Comparison of the upstream DNA sequences of the *SAA1* and *SAA2* genes showed 97% identity of the proximal 750-bp DNA sequences (data not shown). *SAA1* and *SAA2* genes are similarly regulated by a number of proinflammatory cytokines (6). However, differences in the regulation of both genes have been clearly demonstrated in previous studies (5, 26). In the *Saa1*-luc model, we used a 7.7-kb upstream promoter region of the *SAA1* gene to direct luciferase expression. The 5′ end of the *SAA1* promoter region is ~2.1 kb from the ATG translational start codon of *SAA2*. We expect that *SAA1* promoter-driven luciferase expression will serve as a marker for both *SAA1* and *SAA2* production under various inflammatory conditions.

Most of the A-SAAs are synthesized by the *SAA1* and *SAA2* genes. The SAA constitutes the major component of the secondary amyloid plaques that are deposited in the organs as a consequence of chronic inflammatory diseases (27). Conventional methods for monitoring A-SAA synthesis relies mostly on either measuring circulating levels of A-SAA in the serum or mRNA expression. Compared with these methods, in this study we show that measurement of luciferase activity in *Saa1-luc* mice through in vivo imaging provides a convenient alternative for monitoring *SAA1* synthesis under various inflammatory conditions. This approach is sensitive and rapid. The acquisition time for imaging liver luciferase activity in *Saa1-luc* mice can be as little as a fraction of a

Table II. *Luciferase expression*[a]

	Mean ± SE			Significance[b]		
	LPS	Bortezomib + LPS	Saline	LPS vs Saline	Bortezomib + LPS vs Saline	LPS vs Bortezomib + LPS
Liver	$2.0 \pm 0.2 \times 10^6$	$0.2 \pm 0.07 \times 10^6$	209 ± 69	0.05	0.05	0.05
Lung	21,546 ± 3,264	3,920 ± 578	53 ± 14	0.05	0.05	0.05
Intestine	347 ± 54	249 ± 28	19 ± 15	0.05	0.05	0.13
Spleen	1,914 ± 123	1,760 ± 68	1,042 ± 198	0.05	0.05	0.83
Kidney	7,960 ± 2,445	1,751 ± 300	39 ± 6	0.05	0.05	0.05
Brain	351 ± 36	230 ± 16	178 ± 17	0.05	0.13	0.05
Heart	2,334 ± 1,063	470 ± 299	9 ± 5	0.05	0.05	0.13

[a] Luciferase expression (units per milligram of protein) in selected organs from *Saa1-luc* mice (three mice per treatment) 4 h post-i.p. injection with LPS, LPS following pretreatment with bortezomib, or saline.
[b] Significance from nonparametric Mann-Whitney test

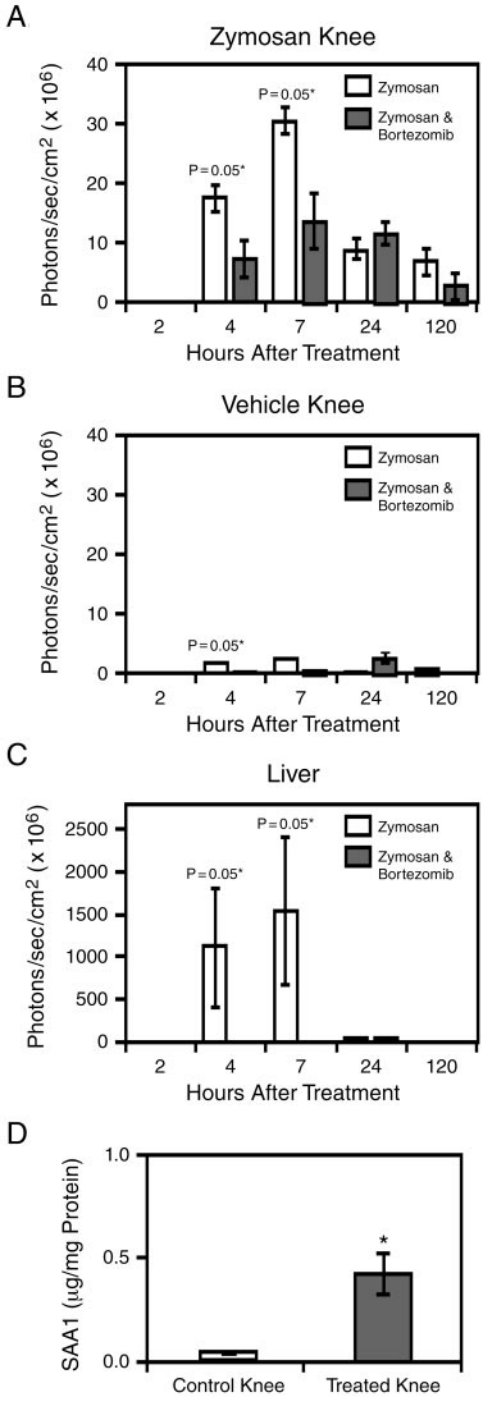

FIGURE 5. Change in luciferase signal over time in female *Saa1-luc* transgenic mice pretreated with bortezomib (1 mg/kg i.v.; $n = 3$) or saline ($n = 3$) 1 h before intra-articular injection of zymosan (300 μg/knee joint) into the right rear knee joint and vehicle (5% glucose in saline) into the left rear knee joint. Mean changes from baseline ± SE are presented. Two of the three bortezomib-pretreated animals were examined at 24 and 120 h. Nonparametric significance levels for the difference between treatment groups were determined by Mann-Whitney *U* test and are presented above the bars. Significant differences ($p = 0.05$) between the zymosan-injected (right) and vehicle-injected (left) rear knee joints are designated with asterisks. *A*, Measurement of luciferase signal from the zymosan-treated knee. *B*, Measurement of luciferase signal from the vehicle-treated knee. *C*, Measurement of luciferase signal from the hepatic region. *D*, Measurement of SAA1 protein production locally in the knee joint after intra-articular injection of either saline or zymosan. Mice ($n = 3$) were killed 6 h after the treatment. SAA1 measurement was performed by ELISA, as described in *Materials and Methods*.

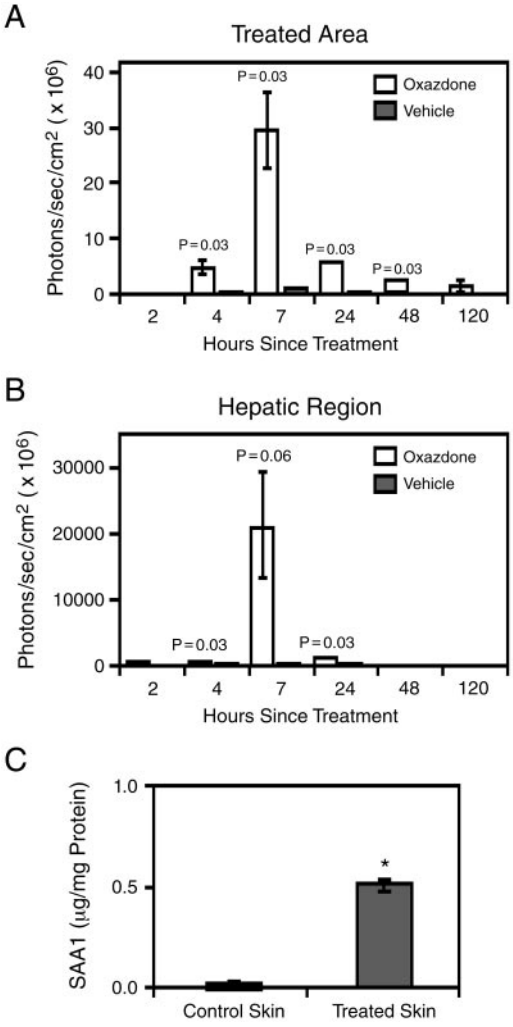

FIGURE 6. *A* and *B*, Change in luciferase signal over time in male *Saa1-luc* transgenic mice receiving oxazolone ($n = 3$) or vehicle ($n = 3$) topically to the shaved thorax area. Mean changes from baseline ± SE are presented. Nonparametric significance levels for the difference between treatment groups were determined by Mann-Whitney *U* test and are presented above the bars. Photons were collected from the treatment area and from the hepatic region. *C*, Measurement of SAA1 protein production locally in oxazolone-treated skin tissue. Mice ($n = 3$) were killed 6 h after the treatment. SAA1 measurement was performed by ELISA, as described in *Materials and Methods*. ∗, Significant difference ($p = 0.05$) between oxazolone-treated skin and vehicle-treated skin (control).

second. In addition, the kinetics as well as the anatomical location of *SAA1* expression can be conveniently studied. This is particularly useful in monitoring local inflammation, such as arthritis and chemical-induced skin toxicity. This is well demonstrated in zymosan-induced arthritis and the oxazolone-induced CHS reaction, which showed both local and systematic induction of luciferase expression after treatment.

Ex vivo analysis of luciferase activity showed that LPS and TNF-α treatments caused induction of luciferase activity in multiple tissues. The most significant luciferase expression under septic conditions triggered by either LPS or TNF-α treatment was in the liver, which showed a few thousand-fold induction, as measured by imaging of live mice and ex vivo measurement of liver luciferase activity. Induction of luciferase activity in the liver after LPS treatment correlated with an increase in *SAA1* mRNA expression. However, we were unable to detect *SAA1* mRNA in other nonhepatic organs from either control or LPS-treated mice. In all

nonhepatic organs of mice treated with either LPS or TNF-α, luciferase activity is <1% and can be as low as 0.004% compared with liver luciferase activity, which suggests that direct detection of endogenous *SAA1* mRNA is not as sensitive as detection of the luciferase reporter. Lower *SAA1* induction in nonhepatic organs indicates that most of the circulating serum amyloids are produced in the liver. In nonhepatic organs such as lungs, kidney, intestine, and heart, LPS or TNF-α treatment caused up to a few hundred-fold induction of luciferase expression, but had little effect on spleen and brain luciferase activity. It is possible that under chronic inflammatory conditions, *SAA1* expression in various tissues may be primed to an induction by local stimuli, and the locally produced *SAA1* may be critical to the process of inflammation and disease progression. Deposition of amyloid plaque, which consists of proteolytically cleaved amyloid fragment, is a major feature in several chronic inflammatory diseases. For example, amyloid accumulates in the brains of patients with Alzheimer's disease, in the pancreata of patients with type II diabetes, and systemically (excluding the brain) in inflammatory diseases, such as rheumatoid arthritis (28). We demonstrated a dramatic inhibitory effect of bortezomib on LPS-induced luciferase expression in liver, kidney, lung, and heart. The inhibition of LPS-induced luciferase expression correlated with a reduction of LPS-induced *SAA1* mRNA expression, as demonstrated in liver tissue. These data suggest that bortezomib or other therapeutic compounds that more specifically target the NF-κB pathway could have a therapeutic effect on chronic inflammatory diseases through suppression of A-SAA production during chronic inflammation. However, it should be noted that bortezomib could be affecting luciferase induction through pathways other than those mediated by NF-κB.

A-SAAs have been found in the synovial fluid recovered from inflamed joints of rheumatoid arthritis patients (15). Both fibroblasts and chondrocytes of the synovial membrane can be induced to produce A-SAA by proinflammatory cytokines such as IL-1, IL-6, and TNF-α (16–19, 29, 30). We clearly demonstrated that local administration of zymosan into the knee joint triggers induction of luciferase expression in that joint, which correlated with an increase in *SAA1* protein in the zymosan-treated knee joint tissue. Transient induction of hepatic signal after zymosan injection into the knee was probably due to the leakage of zymosan into the general circulation and/or an increase in cytokine production at the zymosan-treated knee joint. Compared with the systemic induction in liver, the induction of luciferase expression at the knee joints lasted much longer (see Fig. 5). It is believed that locally synthesized SAA by synovial cells in the inflamed joints plays a key role in the pathogenesis of rheumatoid arthritis (17, 18, 20). SAA can be used as a marker for evaluating the efficacy of therapeutics for rheumatoid arthritis. For example, treatment of rheumatoid arthritis patients with chimeric mAbs to TNF-α significantly improved major clinical end points of the disease process, including a decrease in SAA (31). Transgenic mice overexpressing IL-1R antagonist were protected from collagen-induced arthritis, with reduced level of circulating A-SAA (32). We demonstrated that bortezomib can transiently inhibit the induction of luciferase expression at the knee joint after zymosan administration. This inhibition was not dramatic compared with the inhibition in liver, possibly due to the variation in pharmacokinetics when different tissues are compared. Bortezomib has recently been used as an antineoplastic drug. Whether bortezomib will have a therapeutic effect on rheumatoid arthritis patients needs to be explored.

In summary, we have established a *Saa1-luc* transgenic mouse model and demonstrated that *SAA1* promoter-driven luciferase expression is induced during sepsis, acute arthritis, and CHS reaction. The luciferase induction occurred in multiple organs. Amyloid deposition is a feature in the pathogenesis of a number of chronic inflammatory diseases. Significant SAA induction in liver and other organs by many of the inflammatory agents suggest that locally produced A-SAA may contribute to amyloid deposition in these organs and the pathogenesis of these inflammatory diseases. Pretreatment of mice with bortezomib significantly suppressed LPS- or zymosan-induced *SAA1* promoter-driven luciferase expression, supporting previous findings that the NF-κB pathway may be involved in regulating *SAA1* expression. The *Saa1-luc* mouse is a useful tool not only for tracking various inflammatory processes in vivo, but also for testing the efficacies of therapeutic compounds that are targeted on inflammatory diseases, especially those that involve induction of A-SAA expression.

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Disclosures

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