NF-κB and Not the MAPK Signaling Pathway Regulates GADD45β Expression during Acute Inflammation*

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The GADD45 (growth arrest and DNA damage-inducible) family of genes is involved in the regulation of cell cycle progression and apoptosis. To study signaling pathways affecting GADD45β expression and to examine systematically in vivo the GADD45β expression in tissues following various toxic stresses, we created a transgenic mouse by fusing the GADD45β promoter to firefly luciferase (Gadd45β-luc). In vivo GADD45β expression was assessed by measuring the luciferase activity in the Gadd45β-luc transgenic mouse using a non-invasive imaging system (IVIS® Imaging System, Xenogen Corporation). We found that a number of agents that induce oxidative stress, such as sodium arsenite, CC14, lipopolysaccharide (LPS), or tumor necrosis factor-α, are able to induce luciferase expression throughout the entire animal. In liver, spleen, lung, intestine, kidney, and heart, we observed an induction of luciferase activity after LPS treatment, which correlates with an increase of GADD45β mRNA in these tissues. Processes that induce DNA damage activate the NF-κB signaling pathway. Several inhibitors of the NF-κB signaling pathway, including dexamethasone, thalidomide, and a proteasome inhibitor, bortezomib, showed inhibitory effects on LPS-induced GADD45β expression as indicated by a decrease of the luciferase activity. Northern blot analysis confirmed a broad inhibitory effect of bortezomib on LPS-induced GADD45β mRNA expression in spleen, lung, and intestine. In liver of bortezomib-treated mice, we observed a reverse correlation between the luciferase activity and the GADD45β mRNA level. We speculate that such a discrepancy could be due to severe liver toxicity caused by bortezomib and LPS co-treatment. MAPK inhibitors had transient and inconsistent effects on LPS-induced luciferase expression. Our data are consistent with the notion that NF-κB, but not the MAPK signaling pathways, is involved in the in vivo regulation of GADD45β expression. Thus, NF-κB signaling involves induction of GADD45β expression, which supports the proposed role of GADD45β in protecting cells against DNA damaged under various stress conditions.

GADD45β/MyD118 belongs to the GADD45 nuclear protein family that is expressed ubiquitously in mammalian tissues and is responsive to stimuli that induce DNA damage. Induction of GADD45 expression is involved in the regulation of cell differentiation, cell cycle progression, and apoptosis (1, 2). GADD45 proteins regulate cell cycle and apoptosis by their direct interaction with critical cell cycle and cell survival regulatory proteins, such as proliferating cell nuclear antigen (3) and p21 (WAFl/CIP1) (4). In addition, GADD45 family proteins associate with CDK1 (Cdc2-p34) and inhibit the kinase activity of the CDK1-cyclinB1 complex, which mediate the G2/M cell cycle arrest in response to genotoxic stress (5, 6). Involvement of the GADD45 family proteins has also been implicated in regulating the S-phase checkpoint following UV irradiation (6) and in DNA damage repair (7, 8).

The transcription factor NF-κB family regulates the expression of a variety of proinflammatory and cytotoxic genes (9). In non-stimulated cells, NF-κB proteins are present in the cytoplasm in association with NF-κB-specific inhibitors, such as IκBs (10). Upon stimulation by extracellular inducers, such as TNF-α or interleukin-1β, the IκBs become phosphorylated by IκB kinase complexes and degraded by the proteasome. IκB degradation allows NF-κB to translocate into nuclei and activate the transcription of target genes (11). Activation of NF-κB antagonizes apoptosis or programmed cell death by numerous triggers. Analysis of the GADD45 gene suggests that NF-κB may play a role in regulating GADD45 expression (12). This was later confirmed in a study showing that κB elements of the GADD45β promoter are required for optimal transactivation of GADD45β promoter activity by NF-κB complexes in vitro (13). GADD45β in turn has been shown as a pivotal mediator of the NF-κB signaling pathway (14, 15). GADD45β induction mediates the protective effects of NF-κB against TNF-α and Fas-induced apoptosis (15, 16).

The MAPK signaling cascades are important for many processes in the immune response (17). The stress-responsive p38 and JNK MAPK pathways regulate cell cycle and apoptosis. GADD45 family members (α, β, and γ) are involved in the activation of p38 and JNK pathways (14, 18–20), which in turn participate in the induction of GADD45α expression (21).

To investigate the regulation of GADD45β gene expression in vivo, we developed a transgenic mouse line using the murine GADD45β promoter to direct the expression of a firefly luciferase gene. In conjunction with a highly sensitive IVIS Imaging System, we have applied this model to monitor GADD45β expression non-invasively. The results suggest that the Gadd45β-luc transgenic mouse provides a convenient and sensitive in vivo assay for studying GADD45β expression during acute inflammation.

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‡ The abbreviations used are: GADD, growth arrest and DNA damage-inducible; DEX, dexamethasone; LPS, lipopolysaccharide; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; JNK, c-Jun N-terminal kinase; SAPK, stress-activated protein kinase.
pression in different tissues and for studying the involvement of various signaling pathways in the regulation of GADD45β expression after treatments that lead to inflammatory and toxic stresses.

MATERIALS AND METHODS

Construction of pGadd45β-luc Vector and Generation of Gadd45β-luc Transgenic Mice—A mouse BAC clone containing the mouse GADD45β gene was isolated from a C77 mouse BAC library (Invitrogen). A 10.5-kb promoter fragment containing sequences 5′ to the first ATG for mouse GADD45β gene was obtained by the recombinant cloning method (22) and cloned upstream of the firefly luciferase gene in the pGL3-Basic vector (Promega, Madison, WI). A 0.8-kb human β-globin intron 2 was placed between the GADD45β promoter and the luciferase gene to optimize the luciferase expression in transgenic mice. The transgene cassette was separated from the vector backbone sequences and used for pronuclear injection into CD-1 mouse strain embryos. The transgenic model described in this paper is named CD1-Tg-(Gadd45β-luc)Xen.

Reagents—We purchased bacterial lipopolysaccharide (LPS, from Salmonella abortus equi) dexamethasone, thalidomide, PD098580, sodium arsenite, and carbon tetrachloride from Sigma, recombinant TNF-α from R&D Systems (Minneapolis, MN), Bortezomib (VALCADE, PS-341) from Millennium Pharmaceuticals, Inc (Cambridge, MA), SB203580 from EMD Biosciences, Inc (La Jolla, CA), and SP600125 from A.G. Scientific, Inc (San Diego, CA).

In Vivo Imaging of Luciferase Activity—In vivo imaging was performed using an IVIS Imaging System 100 Series as previously described (23). Gadd45β-luc transgenic mice were anesthetized with isoflurane and injected intraperitoneally with 150 mg/kg luciferin (Bio-synth, A.G., Switzerland). 10 min after the luciferin injection, mice were imaged for 1–60 s. Photons emitted from specific regions were quantified using Living Image® software (Xenogen Corporation). In vivo luciferase activity is expressed as photons/second/cm².

Study of in Vivo GADD45 Gene Regulation Using Gadd45β-luc Transgenic Animals—Gadd45β-luc transgenic mice of 3–6 months of age were intraperitoneally injected with sodium arsenite (1 mg/kg), carbon tetrachloride (0.5 ml/kg), LPS (1 mg/kg), or TNF-α (2 μg/mouse). Control mice were injected with saline. At selected time points after the treatment, mice were intraperitoneally injected with luciferin and imaged 10 min later with the IVIS Imaging System as described above. To test the effect of various compounds on LPS-triggered Gadd45β-luc expression, mice were pretreated with bortezomib (1 mg/kg, intravenous), dexamethasone (10 mg/kg, intraperitoneal), thalidomide (100 mg/kg, intraperitoneal), PD098590 (10 mg/kg, intravenous), SP600125 (20 mg/kg, intravenous), or SB203580 (5 mg/kg, intravenous) 1 h prior to the LPS injection. The luciferase activity was monitored through imaging at various time points over a 24-h period.

For analysis of MAPK activity, a total of 50 μg of liver lysate was loaded onto SDS/PAGE gels. After transfer, the membrane was probed with one of several anti-MAPK antibodies according to the manufacturer’s protocols. The MAPK antibodies used in this study include anti-phospho-p38 MAPK (Thr180/Tyr182) antibody, anti-phospho-p44/42 MAPK antibody, anti-phospho-p44/42 MAPK (Thr202/Tyr204) antibody, anti-p44/42 MAPK antibody, anti-phospho-SAPK/JNK (Thr183/Tyr185) antibody, and anti-SAPK/JNK antibody. All of the MAPK antibodies were purchased from Cell Signaling Technologies (Beverly, MA). The liver lysate was prepared from untreated control mice or LPS-treated mice sacrificed at 4 h after LPS injection (1 mg/kg, intraperitoneal) or LPS-treated mice that were pretreated with one of the MAPK inhibitors (dosed as above) and sacrificed at 4 h after the LPS injection.

Tissue Luciferase Activity—Selected mouse organs were removed and homogenized in 3 volumes of phosphate-buffered saline containing a protease inhibitor mixture (Roche Applied Science) 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, Sunnyvale, CA). Protein concentration was estimated with Bradford reagent (Sigma-Aldrich).

Western Blot Analysis of IxBa Protein Expression—A total of 10 μg of protein was loaded onto SDS/PAGE gels. After transfer, the membrane was probed with a specific anti-IxBa antibody (mouse monoclonal IgG1, Santa Cruz Biotechnology, Santa Cruz, CA). The blots were developed using the ECL® Western blotting detection system (Amersham Biosciences).
mice with sodium arsenite or carbon tetrachloride induced luciferase expression, as demonstrated by bioluminescent images (Fig. 1A). Induction of luciferase expression by sodium arsenite occurred transiently in the whole body. The luciferase induction was evident at 7 h and declined rapidly to base-line value by 24 h. Treatment with carbon tetrachloride, however, caused a more prolonged induction of the luciferase signal, predominantly in the hepatic and abdominal regions. Quantification of the luciferase signal is shown in Fig. 1B. Luciferase signals from the hepatic region and the entire body were induced 2–3-fold by sodium arsenite and carbon tetrachloride. The increase in signal expression was greater with sodium arsenite than carbon tetrachloride after 7 h for both total body and hepatic region signal, whereas the signal was greater in mice treated with carbon tetrachloride than sodium arsenite at 24 and 72 h.

*Induction of GADD45 Expression by LPS and TNF-α—* Gadd45β-luc mice were treated with either LPS or TNF-α. Measurement of the luciferase signal by imaging revealed luciferase signal induction following both treatments (Fig. 2A). The induction occurred in the whole body with high induction in the hepatic region. Luciferase induction peaked 4–7 h after the treatment and was detectable as early as 2 h after the LPS treatment. The signal was reduced sharply by 24 h. Control mice that were injected with saline did not show any induction (results not shown). Another Gadd45β-luc transgenic line (line 96) showed similar basal luciferase expression and response to LPS treatment (Fig. 2B).

Luciferase signal from the hepatic region of the LPS-treated mice was quantified using the IVIS software to produce the data shown in Fig. 3A (upper panel). Female mice had higher mean luciferase signals at base line than males. Both male and female mice showed significant luciferase induction (*p* < 0.05) at 2, 4, 7, and 24 h after LPS treatment as compared with base line (*t* = 0 h). Between the sexes, the fold increase in the luciferase signal after LPS treatment was similar, with a peak of induction observed at 4–7 h (Fig. 3A, right top panel). At the peak, the luciferase signal was induced ~15-fold over base-line levels in both sexes. By 24 h, the luciferase signal declined to 3-fold above the base line. Fig. 3A, bottom panels, displays the kinetics of Gadd45β-luc induction in the hepatic region in mice treated with TNF-α. The luciferase signal was readily detectable at 4 h after TNF-α injection, and the signal remained steady until 7 h. By 24 h, the signal declined to the base line. In both sexes, the luciferase signal was induced 8–11-fold at 4–7 h after the TNF-α injection, which represented a significant increase (*p* < 0.05) from the base line (*t* = 0 h). In separate experiments, we found that mice ranging in age from 2 to 7 months responded in a similar fashion to LPS (data not shown). A similar result was obtained with Gadd45β-luc line 96. As shown in Fig. 3B, mice treated with LPS showed a transient luciferase induction at 4–7 h with a calculated fold of induction between 12- and 16-fold compared with base line (*t* = 0 h).

*Dexamethasone and Thalidomide Inhibited GADD45 Expression by LPS—* We studied the effects of dexamethasone (DEX) and thalidomide on the GADD45β induction by LPS. Gadd45β-luc mice were pretreated with DEX (10 mg/kg, intraperitoneal) or thalidomide (100 mg/kg, intraperitoneal) 1 h before LPS injection. In comparison to the Gadd45β-luc mice that were treated with LPS alone, mice co-treated with either DEX or thalidomide showed significant less luciferase induction (Fig. 4). The inhibition was evident in the hepatic region only. Quantification of the luciferase induction over 24 h after the treatment is shown in Fig. 5. Pretreatment with either DEX or thalidomide caused approximate a 50% inhibition of the hepatic luciferase signal in both males and females when compared with the LPS-treated positive control mice.

*Effect of the MAPK Inhibitors on GADD45β Induction by LPS—* We studied the effect of three MAPK inhibitors, PD098059, SB203580, and SP600125, on LPS-induced GADD45β expression. These inhibitors have been shown to inhibit ERK, p38, and JNK MAPK signaling pathways, respectively. The bioluminescent imaging data and the quantification of the luciferase signal from the hepatic region are shown in Fig. 6, A and B, respectively. PD098059 did not have any significant effect on LPS-induced luciferase expression in the
Bortezomib Inhibited LPS-induced GADD45β Expression—

Using the Gadd45β-luc model, we tested the effect of a proteasome inhibitor, bortezomib, on LPS-induced GADD45β expression in vivo. Bortezomib suppressed IxB degradation by the proteasome, thus inhibiting NF-xB transcriptional activity. Gadd45β-luc mice were pretreated with bortezomib (1 mg/kg, intravenous) 1 h before LPS treatment, and the bioluminescent images are presented in Fig. 7A. In both males and females, we observed a significant inhibition of LPS-induced luciferase expression by bortezomib in the whole body. The most dramatic inhibition occurred in the hepatic region. Quantification of the signal from the hepatic region is shown in Fig. 7B. Inhibition of LPS-induced luciferase activity by bortezomib was observed at 2 (p = 0.02), 4, and 7 h (p = 0.004 in males; p = 0.02 in females at all time points). The calculated fold of increase from the base line is shown in Fig. 7C. At the peak induction of 4–7 h, the LPS-treated mice had 14–17-fold increase of luciferase signal from the base line, whereas the bortezomib co-treated mice had 0.2–2-fold induction. Bortezomib-treated mice of both genders appeared ill within 24 h following LPS treatment. All of the female mice and most of the male mice did not survive by 24 h after bortezomib and LPS co-treatment.

Analysis of Tissue Luciferase Activity and GADD45β mRNA Expression—Table I displays the luciferase activity in selected organs in Gadd45β-luc mice. In saline-treated control mice, ex vivo luciferase activity was detected in all of the dissected organs. Both male and female controls showed a similar pattern of luciferase expression in these tissues. The luciferase activity of controls was the highest in brain, spleen, and lung and lowest in intestine and intermediate in liver, heart, and kidney. 4 h following LPS injection, luciferase activity was induced in all of the examined organs, except in the brain. In the LPS-treated mice, the luciferase activity was the highest in hepatic region. Both SP600125 and SB203580 showed significant inhibitory effects at 4 h (p = 0.05), but not at 2 or 7 h, on luciferase induction when compared with LPS-treated control mice (Fig. 6, A and B). Ex vivo measurement of the luciferase activity from the liver lysate failed to show any significant effect by SB203580 (p = 0.13) or PD098059 (p = 0.28) or SP600125 (p = 0.51) on LPS-induced luciferase activity in the liver tissue harvested 4 h after LPS treatment (Fig. 6C).

We next analyzed the MAPK activation using specific antibodies against phosphorylated p38, ERK (p44/42), or SAPK/JNK MAPK. As shown in Fig. 6D, p38 MAPK was already activated in the liver tissue of the control mice. This activity was not regulated by either LPS or SB203580 treatment (top panel). The ERK MAPK activity is also present in the liver tissue of control mice at variable levels. There was no consistent induction of ERK protein phosphorylation by LPS or inhibition by the specific inhibitor PD098059 (middle panel). The SAPK/JNK MAPK activity was not detectable in the control liver tissue. This activity was induced by LPS, and the induction was totally inhibited by the inhibitor SP600125.
liver, spleen, and lung, lowest in the brain, and intermediate in intestine, heart, and kidney. With the exception of the brain, LPS-treatment significantly increased luciferase expression in all of the tissues in both male \((p = 0.006)\) and female \((p = 0.05)\) mice. Again, male and female LPS-treated mice showed a similar pattern of increased luciferase expression across all of the organs examined. As calculated from the mean ± S.D. of the saline-injected mice, LPS treatment in male and female mice caused luciferase induction of 60- and 29-fold, respectively, in the liver, 20- and 15-fold in the spleen, 45- and 66-fold in the small intestines, 7- and 6-fold in the heart, 15- and 15-fold in the kidney, and 9- and 10-fold in the lung.

We examined the effect of bortezomib on the LPS-induced GADD45β expression in selected tissues. Gadd45β-luc mice were pretreated with bortezomib (1 mg/kg, intravenous) 1 h prior to the LPS treatment. Selected organs were removed at 4 h after the LPS treatment. The data are presented in Table I. In comparison with LPS-treated mice, mice pretreated with bortezomib showed significant inhibition of luciferase induction in liver, spleen, and lung.
in both males and females ($p < 0.05$). In kidney and heart, the inhibition was observed in both genders but this was statistically significant in heart only in females ($p < 0.05$) and in kidney in males ($p < 0.01$). In intestine and brain, bortezomib did not significantly affect the LPS-induced luciferase activity. When compared with luciferase activity in the organs of the LPS-treated mice, bortezomib treatment caused an 80% inhibition of luciferase activity in the liver and a 30–70% inhibition of luciferase activity in all of the rest of the non-brain tissues.

We further attempted to establish the correlation between the luciferase activity and the $GADD45\beta$ mRNA expression in selected tissues harvested from female $Gadd45\beta$-luc transgenic mice (Fig. 7D). In untreated control mice, $GADD45\beta$ mRNA expression was detectable in lung and brain only, which are also among the organs that expressed the highest luciferase activity. Following LPS treatment, we observed an induction of $GADD45\beta$ mRNA expression in all of the tissues examined. Lung, kidney, and spleen had the highest $GADD45\beta$ mRNA expression compared with other tissues that correlated with the luciferase activity in these tissues. However, in the liver, LPS only slightly increased $GADD45\beta$ mRNA level. Pretreatment with bortezomib inhibited the induction of $GADD45\beta$ mRNA by LPS in spleen, intestine, and lung. Bortezomib did not affect LPS-induced $GADD45\beta$ mRNA expression in kidney. These results are in agreement with the luciferase activity measurements shown in Table I. Unexpectedly, we observed an increase of $GADD45\beta$ mRNA level in the bortezomib pretreated liver in comparison with LPS-treated liver, even though the luciferase activity was inhibited by bortezomib.

The Regulation of $GADD45\beta$ Expression by LPS and Bortezomib Correlates with Change of $I\kappaB$ Protein Level in Multiple Tissues—To provide further evidence that the NF-$\kappaB$ signaling pathway is involved in the regulation of $GADD45\beta$ expression, we analyzed the $I\kappaB\alpha$ protein level by Western blot analysis in various tissues of control and LPS-treated mice with and without bortezomib pretreatment. Basal levels of $I\kappaB\alpha$ protein were observed in all of the tissues examined (Fig. 8). After LPS treatment, $I\kappaB\alpha$ protein level was decreased in all
of the tissues, which corresponds with previous reports that LPS treatment causes degradation of IκBα protein. Pretreatment with bortezomib increased IκBα protein in spleen, liver, lung, intestine, and brain compared with the tissues of LPS-treated mice; supporting the hypothesis that bortezomib suppressed GADD45α expression by inhibiting IκBα degradation and NF-κB activation. In kidney and heart, bortezomib did not inhibit LPS-induced IκBα protein degradation. The effect of LPS and bortezomib on IκBα protein level is largely matched with the regulation of GADD45α-mediated luciferase activity in all of the tissues examined. The regulation of IκBα protein level and GADD45α-luc expression by LPS and bortezomib support the involvement of the NF-κB signaling pathway in the regulation of GADD45α expression.

**DISCUSSION**

In this study, we used a Gadd45α-luc transgenic mouse model to study the expression and regulation of the GADD45α gene in vivo under a variety of stress conditions. The luciferase activity was the highest in lung and brain of untreated mice, which matches the higher level of GADD45α mRNA expression in these tissues. After LPS treatment, we observed an induction of GADD45α mRNA expression in all of the tissues examined, which is indicated by an increase of luciferase activity in all of the tissues, except the brain. Our data support the involvement of NF-κB signaling in the regulation of GADD45α induction by LPS. This conclusion is supported by the observation that LPS-treated mice had less IκBα protein in various tissues compared with the control tissues. In addition, compounds that have been demonstrated to inhibit NF-κB including dexamethasone (24) and thalidomide (25) suppressed the luciferase induction by LPS.

Bortezomib is a proteasome inhibitor that suppresses the degradation of IκBα and the transcriptional activity by the NF-κB complex (26). Bortezomib has efficacy against a broad range of tumors, including myeloma, chronic lymphocytic leukemia, prostate cancer, pancreatic cancer, and colon cancer.
activity in these tissues. The increase of \( \text{IxB} \) protein level in spleen, lung, and intestine of bortezomib-pretreated mice compared with mice treated with LPS alone support the suppression of \( \text{GADD45B} \) expression mediated through inhibition of NF-\( \kappa \)B activity. In addition, bortezomib did not inhibit LPS-induced \( \text{GADD45B} \) mRNA expression in kidney, which was consistent with both the lack of inhibition of the luciferase activity as well as the lack of regulation of \( \text{IxB} \) protein level by bortezomib.

In the liver tissue, we observed an increase of \( \text{GADD45B} \) mRNA level in the bortezomib-pretreated mice compared with mice treated with LPS alone. This contradicts the observation that bortezomib inhibits LPS-induced luciferase activity in the liver. This discrepancy could be due to severe liver toxicity caused by bortezomib and LPS co-treatment. Under such a severe toxic condition, the luciferase reporter failed to represent the endogenous gene expression. Furthermore, the increase of \( \text{GADD45B} \) mRNA level in the liver of bortezomib pretreated mice suggested that an NF-\( \kappa \)B-independent mechanism might exist for induction, because the liver \( \text{IxB} \) protein level was higher in these mice compared with the liver tissue of the LPS-treated mice.

Involvement of the MAPK signaling pathway is implicated for the regulation of \( \text{GADD45} \alpha \) expression (21). However, whether the MAPK signaling pathways are involved in the regulation of \( \text{GADD45B} \) expression has not been extensively studied. Using the \( \text{GADD45B-luc} \) mice, we demonstrated that LPS treatment that induced \( \text{GADD45B} \) expression and luciferase expression in the liver did not affect ERK (p44/p42) or p38 phosphorylation. Treatment of the mice with specific inhibitor PD98059 did not affect the basal ERK (p44/p42) activity or the LPS-induced luciferase activity, indicating that the ERK (p44/p42) MAPK was not involved in the regulation of \( \text{GADD45B} \) expression. Treatment of the mice with the specific inhibitor SB203580 failed to demonstrate any consistent effect on LPS-induced luciferase activity. Further analysis showed that SB203580-treated mouse liver tissue had a similar level of p38 kinase activity compared with the control or LPS-treated mouse liver tissues. Thus, p38 was not involved in the regulation of \( \text{GADD45B} \) expression either. The SAPK/JNK activity in the liver tissue of control mice was undetectable. After LPS treatment, we detected an induction of the SAPK/JNK activity, which was totally blocked by the specific inhibitor SP600125. The regulation of SAPK/JNK activity did not consistently affect

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**Table I**

*Ex vivo luciferase expression (unit/µg protein) in selected organs from Gadd45B-luc mice*

|       | Mean ± S.E. |       |       |       |
|-------|------------|-------|-------|-------|
|       | Male       | Female| LPS   | Bortezomib|
| Liver | 19.0 ± 5.5 | 27.2 ± 1.5 | 1139.2 ± 103.5* | 803.0 ± 60.1b | 242.1 ± 51.6c | 153.5 ± 24.9\( ^{a,b} \) |
| Brain | 106.9 ± 25.3 | 65.9 ± 3.6 | 108.0 ± 20.2 | 81.0 ± 25.5 | 125.7 ± 20.4 | 108.0 ± 6.8b |
| Spleen | 61.9 ± 12.1 | 60.9 ± 3.2 | 1255.0 ± 300.5* | 885.6 ± 133.4b | 546.6 ± 74.9d | 419.6 ± 87.6e |
| Small intestine | 8.7 ± 4.1 | 2.3 ± 0.7 | 394.5 ± 149.2b | 149.0 ± 21.5b | 127.8 ± 39.2b | 94.7 ± 9.5b |
| Heart | 21.8 ± 6.7 | 21.6 ± 1.8 | 160.3 ± 35.6c | 129.0 ± 7.1b | 91.4 ± 12.0b | 77.7 ± 11.0d |
| Kidney | 15.1 ± 2.0 | 22.7 ± 1.4 | 234.1 ± 29.8c | 343.2 ± 26.1b | 113.8 ± 21.6c | 248.0 ± 45.7b |
| Lung | 85.8 ± 30.8 | 45.9 ± 3.5 | 753.4 ± 121.0b | 480.1 ± 101.3b | 220.7 ± 15.8c | 201.8 ± 15.6d |

*Significantly difference from controls at \( p = 0.006 \).
*Significantly difference from controls at \( p = 0.05 \).
*Significantly difference from LPS-treated mice at \( p = 0.004 \).
*Significantly difference from LPS-treated mice at \( p = 0.05 \).
*Significantly difference from controls at \( p = 0.009 \).
*Significantly difference from LPS-treated mice at \( p = 0.01 \).
*Significantly difference from controls at \( p = 0.03 \).

(27). In the analysis of \( \text{GADD45B} \) mRNA expression in various tissues from bortezomib and LPS co-treated mice, we observed a divergent effect of bortezomib on LPS-induced \( \text{GADD45B} \) mRNA expression. LPS-induced \( \text{GADD45B} \) mRNA expression was inhibited in spleen, lung, and intestine, increased in liver, and unaffected in kidney and brain by bortezomib. These differences could be due to the variation in the pharmacokinetics of bortezomib in these tissues. The inhibition of LPS-induced \( \text{GADD45B} \) mRNA expression in spleen, lung, and intestine by bortezomib certainly correlated with a decrease of luciferase expression.
GADD45β promoter-mediated luciferase expression, thus ruling out the involvement of the SAPK/JNK signaling pathway in the regulation of the GADD45β expression. Our data do not support a significant role of the any of the MAPK signaling pathways in the regulation of GADD45β induction by LPS.

Arsenic and CCl4 have been commonly used as stress-inducing agents in animal models. Exposing animals to these chemicals induces carcinogenicity in various organs due to the genotoxic effect of these compounds (28, 29). In the Gadd45β-luc mice, the toxicity caused by these compounds, as well as by other inflammatory stress-inducing agents such as LPS and TNFα, can be monitored through the induction of luciferase activity. Induction of luciferase activity in the whole body by these agents suggests that toxicity occurred systemically after treatment. Thus, Gadd45β-luc mice may be a useful toxicity model. The utility of this model could be expanded to chronic disease conditions, such as tumor development.

In summary, we have established a Gadd45β-luc transgenic mouse model and successfully applied it to study in vivo expression and regulation of the GADD45β gene. Luciferase expression in the Gadd45β-luc mice may serve as a sensor for non-invasively detecting oxidative stress and DNA damage in live animals. Further validation of toxicology applications may enable the Gadd45β-luc mice to become a convenient tool for accessing in vivo risk to xenobiotic exposure and for predicting development of disease.

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REFERENCES

1. Abdollahi, A., Lord, K. A., Hoffman-Liebermann, B., and Liebermann, D. A. (1991) Oncogene 6, 165–167
2. Fornace, A. J., Jr., Jackman, J., Hollander, M. C., Hoffman-Liebermann, B., and Liebermann, D. A. (1992) Annu. N. Y. Acad. Sci. 668, 139–153
3. Smith, M. L., Chen, I. T., Zhan, Q., Bae, I., Chen, C. Y., Gilmer, T. M., Kastan, M. B., O'Connor, P. M., and Fornace, A. J., Jr. (1994) Science 266, 1766–1780
4. Kearsey, J. M., Costes, P. J., Prescott, A. R., Warnbrick, E., and Hall, P. A. (1995) Oncogene 11, 1675–1683
5. Zhan, Q., Antinore, M. J., Wang, X. W., Carrier, F., Smith, M. L., Harris, C. C., and Fornace, A. J., Jr. (1999) Oncogene 18, 2892–2900
6. Vairapandi, M., Balliet, A. G., Hoffman, B., and Liebermann, D. A. (2002) J. Cell. Physiol. 192, 327–338
7. Smith, M. L., Ford, J. M., Hollander, M. C., Bortnick, R. A., Amundson, S. A., Seo, Y. B., Deng, C. X., Hanawalt, P. C., and Fornace, A. J., Jr. (2000) Mol. Cell. Biol. 20, 3705–3714
8. Vairapandi, M., Balliet, A. G., Fornace, A. J., Hoffman, B., and Liebermann, D. A. (1996) Oncogene 15, 2579–2594
9. Karin, M., and Delhase, M. (1996) Proc. Natl. Acad. Sci. U. S. A. 95, 9067–9069
10. Baldwin, A. S., Jr. (1996) Annu. Rev. Immunol. 14, 649–683
11. Li, Q., and Verma, I. M. (2002) Nat. Rev. Immunol. 2, 975
12. Balliet, A. G., Hattonn K. S., Hoffman, B., and Liebermann, D. A. (2001) DNA Cell Biol. 20, 239–247
13. Jin, R., De Smaele, E., Zazzeroni, F., Nguyen, D. U., Papa, S., Jones, J., Cox, C., Gelinas, C., and Franzenso, G. (2002) DNA Cell Biol. 21, 491–503
14. Papa, S., Zazzeroni, F., Bubici, C., Jayawardena, S., Alvarez, K., Matsuda, S., Nguyen, D. U., Pham, C. G., Nelseb, A. H., Melis, T., Smacile, E. D., Tang, W. J., D’Adami, L., and Frangessa, G. (2004) Nat. Cell Biol. 6, 146–153
15. De Smacle, E., Zazzeroni, F., Papa, S., Nguyen, D. U., Jin, R., Jones, J., Cong, R., and Frangesso, G. (2001) Nature 414, 308–313
16. Zazzeroni, F., Papa, S., Algeciras-Schimminich, A., Alvarez, K., Melis, T., Bubici, C., Majezski, N., Hay, N., De Smacle, E., Peter, M. E., and Frangesso, G. (2003) Blood 102, 3270–3279
17. Dong, C., Davis, R. J., and Flavell, R. A. (2002) Annu. Rev. Immunol. 20, 55–72
18. Takekawa, M., and Saito, H. (1998) Cell 95, 521–530
19. Yang, J., Zhu, H., Murphy, T. L., Ouyang, W., and Murphy, K. M. (2001) Nat. Immunol. 2, 157–164
20. Takekawa, M., Tatebayashi, K., Itoh, F., Adachi, M., Imai, K., and Saito, H. (2002) EMBO J. 21, 6473–6482
21. Tong, T., Fan, W., Zhao, H., Jin, S., Fan, F., Blanck, P., Alomo, I., Rajasekaran, B., Liu, Y., Holbrook, N. J., and Zhan, Q. (2001) Exp. Cell Res. 269, 64–72
22. Lee, E. C., Yu, D., Martinez de Velasco, J., Tressarillo, L., Swing, D. A., Court, D. L., Jenkins, N. A., and Copeland, N. G. (2001) Genomics 73, 56–66
23. Contag, P. R., Odom, L. N., Stevenson, D. K., and Contag, C. H. (1998) Nat. Med. 4, 245–247
24. Spiecker, M., Darius, H., and Liao, J. K. (2000) J. Immunol. 164, 3316–3322
25. Keifer, J. A., Guttridge, D. C., Ashburner, B. P., and Baldwin, A. S., Jr. (2001) J. Biol. Chem. 276, 22382–22387
26. Tan, C., and Waldmann, T. A. (2002) Cancer Res. 62, 1083–1086
27. Adams, J., and Kaufman, M. (2004) Cancer Invest. 22, 304–311
28. Gehel, T. (2000) Toxicology 144, 155–162
29. Weber, L. W., Boll, M., and Stampfl, A. (2003) Crit. Rev. Toxicol. 33, 105–136