Modulation of glucocorticoid receptor expression, inflammation, and cell apoptosis in septic guinea pig lungs using methylprednisolone

Koki Kamiyama,1,2 Naoyuki Matsuda,1 Seiji Yamamoto,1 Ken-ichi Takano,1 Yasuo Takano,3 Hiromi Yamazaki,1 Shun-ichiro Kageyama,1 Hiroki Yokoo,1 Takuya Nagata,2 Noboru Hatakeyama,4 Kazuhiro Tsukada,2 and Yuichi Hattori1

1Department of Molecular and Medical Pharmacology, 2Department of Surgery, 3Department of Diagnostic Pathology, and 4Department of Anesthesiology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan

Submitted 6 November 2007; accepted in final form 2 October 2008

Kamiyama K, Matsuda N, Yamamoto S, Takano K, Takano Y, Yamazaki H, Kageyama S, Yokoo H, Nagata T, Hatakeyama N, Tsukada K, Hattori Y. Modulation of glucocorticoid receptor expression, inflammation, and cell apoptosis in septic guinea pig lungs using methylprednisolone. Am J Physiol Lung Cell Mol Physiol 295: L998–L1006, 2008. First published October 3, 2008; doi:10.1152/ajplung.00459.2007.—The use of glucocorticoids for treatment of sepsis has waxed and waned during the past several decades, and recent randomized controlled trials have evoked a reassessment of this therapy. Most glucocorticoid actions are mediated by its specific intracellular receptors (GRs). Thus we initially evaluated whether sepsis and high-dose corticosteroid therapy can regulate guinea pig pulmonary expression of GRs: active receptor, GRα, and dominant negative receptor, GRβ. Sepsis induction by LPS injection (300 μg/kg ip) increased mRNA and protein levels of GRs and increased protein expression of GRβ in lungs. High-dose methylprednisolone (40 mg/kg ip), administered simultaneously with LPS, markedly potentiated the decrease in GRα expression but slightly affected the increase in GRβ expression. Consequently, this led to a significant reduction in GRs nuclear translocation. Nevertheless, methylprednisolone treatment strongly eliminated LPS induction of NF-κB activity, as determined by NF-κB nuclear translocation and by gel mobility shift assays. Furthermore, the LPS-induced increase in inflammatory cells in bronchoalveolar lavage fluid was blunted by administration of the corticosteroid. On the other hand, immunofluorescent staining for cleaved caspase-3 showed a marked increase in this proapoptotic marker in lung sections, and terminal deoxynucleotidyl transferase dUTP-mediated nick-end labeling (TUNEL) represented an enhanced appearance of cell apoptosis in lungs and spleen when methylprednisolone was given together with LPS. Cell apoptosis is now considered to play a role in the pathogenesis of septic syndrome. We thus suggest that the action of glucocorticoids at high doses to accelerate sepsis-induced cell apoptosis may overwhelm their therapeutic advantages in septic shock.

lung inflammation; nuclear factor-κB

DESPITE ADVANCES IN SUPPORTIVE CARE, severe sepsis and septic shock continue to be the leading cause of death in critically ill patients (33). A major factor contributing to the high morbidity and high mortality of septic shock is the lack of an effective treatment (46). Corticosteroids have been used for treatment of sepsis since the 1950s. Their fundamental roles in stress response to infection and increasing knowledge of the anti-inflammatory and immunosuppressive pharmacodynamic profile have been the rationale for its use in sepsis trials for decades. However, the current trend favors the use of corticosteroids, especially in the setting of septic shock and relative adrenal insufficiency (2, 11). Several randomized controlled trials suggest that prolonged administration of “low” doses of corticosteroids in patients with sepsis and acute respiratory distress syndrome (ARDS) improves clinical outcomes (2, 7, 8, 38). These results may be in agreement with the concept of impaired adrenocortical reserve in septic shock (9, 48, 50). Such a beneficial effect was not seen in trials using high-dose methylprednisolone. The harmful effect of high-dose methylprednisolone may be due to immunosuppression and increased incidence of secondary infections (39). Moreover, quite recent clinicopneumological studies have not provided any support for the routine use of methylprednisolone for treatment of ARDS (44, 51).

Potential molecular mechanisms underlying the harmful effect of high-dose corticosteroid therapy on the outcome of sepsis are insufficiently understood. We designed a study seeking to highlight the molecular changes in lung tissues associated with high-dose corticosteroid therapy in an in vivo guinea pig model of endotoxin challenge. In this study, methylprednisolone was used for corticosteroid therapy, since it was the most frequently used glucocorticoid at high doses in patients with sepsis and/or ARDS because of its high renal clearance (38, 44, 51).

The apheliotropic actions of glucocorticoids in the cell are mediated through an intracellular glucocorticoid receptor (GR) (56). In the nucleus, GR binds as a homodimer to glucocorticoid response elements (GREs) on the DNA to transactivate target genes, which may be positive or negative, either activating or repressing transcription (5, 32). This mode of GR action is generally considered to be responsible for GR-mediated side effects. Another mechanism of GR action is currently thought to be important for most of the GR anti-inflammatory functions, which is based on the ability of liganded GR monomers to interact directly with other proinflammatory transcription factors, such as NF-κB, thereby repressing or potentiating their activity (17). GR gene transcription may be crucially changed after induction of sepsis, although conflicting results have been reported concerning the effect of sepsis on GR expression (29, 49, 52, 53, 54). Therefore, the first aim of the present study was to investigate the effect of high-dose corticosteroid therapy on GR expression in septic guinea pig lungs.

L998 1040-0605/08 $8.00 Copyright © 2008 the American Physiological Society http://www.ajplung.org
using immunohistochemistry, Western blotting, and RT-PCR analyses.

Glucocorticoid-mediated regulation of apoptosis has been described in a number of different cells and tissues, including thymocytes and neurons (4, 45). Most of these effects can contribute to the immunosuppressive and antineoplastic action of glucocorticoids but may be involved in their unwanted effects, which would limit their therapeutic use. Recently, cell apoptosis has been implicated playing an important role in the development of organ failure and mortality associated with sepsis (42). In this setting, lymphocytes and parenchyma cells, including intestinal and lung epithelial cells, as well as vascular endothelial cells undergo apoptotic death (20, 25, 60). Therefore, the second aim was to assess whether this high-dose corticosteroid therapy can significantly affect lung apoptosis in the guinea pig septic model.

MATERIALS AND METHODS

Animals and treatments. This study was conducted in accordance with the National Institutes of Health guidelines on the use of laboratory animals and with approval of the Animal Care and Use Committee of the University of Toyama. Male Hartley guinea pigs, weighing 300–400 g, were housed in an environment with controlled temperature (22–23°C), constant humidity (55–60%), and a daily 12-h light-dark cycle. Standard chow and drinking water were provided ad libitum. A period of at least 7 days was allowed for acclimatization before any experimental manipulation was undertaken.

The guinea pigs were divided into two groups in a random block design and injected intraperitoneally with LPS (300 µg/kg; Escherichia coli 055; List Biological Laboratories, Campbell, CA) or an equivalent volume of sterile saline. Intraperitoneal treatment with methylprednisolone (40 mg/kg) was performed contemporaneously to an equivalent volume of sterile saline. After 24 h from LPS or saline injection, the animals were killed by an overdose of pentobarbital.

Western blot analysis. After being removed and rinsed in sterilized PBS on ice, lungs were homogenized and then centrifuged at 600 g to pellet any insoluble material. The protein concentration of supernatant was determined using a BCA Protein Assay Kit (Pierce; Rockford, IL). When the nuclear extract was used for the sample, it was prepared from lung tissues as demonstrated in our previous study with minor modification (34). Samples (10–20 µg of protein) were run on 7.5% SDS-polyacrylamide gels. Blotting procedures, chemiluminescent detection, and densitometric analysis were carried out as previously described by our laboratory (34–36). Membranes were probed with anti-GR (Calbiochem, San Diego, CA), anti-GRβ (Calbiochem), anti-NF-κB p65 (Cell Signaling Technology, Danvers, MA), anti-lamin B (PROGEN Biotechnik, Heidelberg, Germany), and anti-actin (GeneTex, San Antonio, TX).

Immunofluorescence and confocal analysis. As previously described (34), the trachea was cannulated, and the lungs were gently inflated-fixed en bloc with 4% buffered formalin solution. Inflation-fixed lungs were harvested, postfixed in 4% paraformaldehyde overnight, immersed in sucrose solutions, dipped into OCT compound (Sakura Finetechinal, Tokyo, Japan), and frozen at −20°C. The tissues were then sectioned at a thickness of 30 µm and air dried. For immunohistochemical determination of target molecules, the tissue sections were exposed to the fluorescent antibody after overnight incubation with the primary antibody, according to the method in our (36) previous study with minor modification. The following commercially available antibodies were used: anti-GR antibody, anti-cytokeratin antibody (Sigma Chemical, St. Louis, MO), anti-surfactant protein C (SP-C) antibody (Chemicon, Temecula, CA), anti-von Willebrand factor (vWF) antibody (Chemicon), anti-CDF8 antibody (AbD Serotec, Kidlington, Oxford, United Kingdom), anti-α-smooth muscle actin (αSMA) antibody (Sigma Chemical), and anti-cleaved caspase-3 antibody (Cell Signaling Technology). Zenon labeling technology (Molecular Probes, Eugene, OR) was used in experiments where multiple primary antibodies of the same type were present. The nucleus was counterstained with Hoechst 33258 (Nacalai Tesque, Kyoto, Japan). Immunofluorescent images were observed under Olympus BX51 fluorescence microscope or Leica TCS-SP5 confocal system.

RNA extraction and RT-PCR. Total RNA was isolated from lung tissues with the use of a TRI reagent (Sigma Chemical) according to the manufacturer’s instructions. RNA was reverse-transcribed to cDNA using random hexamers. cDNA was amplified with primers specific for glucocorticoid receptor (GR), GRβ, GRα, NF-κB p65, lamin B, αSMA, ACTIN, GAPDH, and G3PDH as previously described (34). A representative example of a RT-PCR analysis is shown. Values are means ± SE; n = 4. *P < 0.05 vs. control; #P < 0.05 vs. LPS alone.

Fig. 1. Effect of treatment with methylprednisolone (GC; 40 mg/kg ip) on glucocorticoid receptor-α (GRα) expression in lungs from control and LPS-challenged guinea pigs. In A and B, GRα protein expression levels were assessed in the total and nuclear fractions, respectively. In the top trace of each panel, typical Western blots are shown. Actin served as loading control, and lamin B as a nuclear membrane marker. C, GRα mRNA and β-actin mRNA were evaluated by RT-PCR as described in MATERIALS AND METHODS, and a representative example of ethidium bromide-stained agarose gels is shown. Values are means ± SE; n = 4. *P < 0.05 vs. control. #P < 0.05 vs. LPS alone.
cDNA, and PCR was performed using a Takara RNA PCR kit (Takara Shuzo, Ohtsu, Japan) as described in the manufacturer’s manual. Expression of the GR gene was monitored by PCR with 5′-CCCTG-GATGATGACCAATG-3′ (sense) and 5′-AGTAGTTTTTGATGCTCC-3′ (antisense). The PCR-amplified product was analyzed by agarose gel electrophoresis. The internal standard used was the ubiquitously expressed housekeeping gene β-actin, which was amplified with 5′-ACTCTCCACCTCCAGCA-3′ and 5′-AAAGCCAT-TGCCAATCTCATC-3′ primers producing a 176-bp fragment.

Nuclear extraction and EMSA. Nuclear protein extracts from freshly isolated lungs were obtained with a commercially available nuclear extraction kit (Sigma Chemical) as described in the manufacturer’s manual. For the detection of NF-κB DNA binding activity, double-stranded oligonucleotides with consensus sequences of NF-κB (5′-AGT TGA GGG GAC TTT CCC AGG C-3′; Santa Cruz Biotechnology, Santa Cruz, CA) were end labeled with [32P]ATP (4,500 Ci/mmol at 10 Ci/µl; MP Biomedicals, Solon, OH) as recommended by the manufacturer (Takara Shuzo). The DNA binding reaction was conducted, and then samples were loaded onto a 5% polyacrylamide gel and run at 100 V for 60 min after adding bromphenol blue and xylene cyanol. Gels were then dried under a vacuum and subjected to autoradiography.

Cell counting in bronchoalveolar lavage fluid. Under anesthesia with pentobarbital, the lungs were gently lavaged two times with 5 ml of PBS containing heparin, and lavage fluid was pooled. The recovery ratio of pooled lavage fluid ranged from 81% to 88% and did not differ significantly among the groups. The bronchoalveolar lavage fluid (BALF) was then centrifuged at 1,000 g for 15 min to separate cells from the supernatant. Cells were resuspended in PBS and counted with a hemocytometer. Slides were stained with May-Giemsa stain, and cell differential was tabulated using light microscopy at 400 magnification.

Apoptotic cell detection. Terminal deoxynucleotidyl transferase (Tdt) dUTP-mediated nick-end labeling (TUNEL) was performed using the ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit (Chemicon) according to the manufacturer’s instructions. In brief, paraffin sections (4-µm thick) were digested with 20 µg/ml proteinase K (Takara Shuzo) for 10–15 min at room temperature and reacted with Tdt enzyme for 60 min at 37°C. The sections were then incubated with anti-digoxigenin conjugate at room temperature for 30 min.
and actin are shown in the Fig. 4. Effect of treatment with GC (40 mg/kg ip) on GRβ expression in lungs from control and LPS-challenged guinea pigs. GRβ protein expression levels were assessed in the total and nuclear fractions. Typical Western blots of GRβ and actin are shown in the top trace. Notably, GRβ was undetectable in the nuclear fraction from any group. Values are means ± SE; n = 4. *P < 0.05 vs. control; #P < 0.05 vs. LPS alone.

followed by incubation with diaminobenzidine solution and counterstained with methyl green. Apoptotic cells were observed in cross-section in randomly selected microscopic fields at a final magnification of ×400.

Serum cortisol assay. Guinea pig blood was collected into microtubes and centrifuged to obtain serum. Serum cortisol assays were performed with the use of the ADVIA Centaur Assay (Bayer Medical, Tokyo, Japan) in accordance with the manufacturer’s guidelines. This method showed no cross-reactivity with other steroid hormones such as aldosterone, progesterone, and testosterone, and the assay limit of sensitivity was 0.2 μg/dl with an interassay variation of ±10%.

Statistical analysis. Data are expressed as means ± SE. Statistical assessment of the data was made by one-way ANOVA with repeated measurements followed by Bonferroni multiple-comparison test when appropriate. A P value of <0.05 was considered to be statistically significant.

RESULTS

Effect of endotoxin on serum cortisol. At 3 h after LPS administration to the guinea pig, the serum cortisol level was elevated from 51 ± 4 (n = 10) to 142 ± 40 μg/dl (n = 6). This elevation at the 3-h time point was variable from animal to animal but was statistically significant (P < 0.01). Serum cortisol increased approximately sixfold from baseline at 6 h after endotoxin (309 ± 9 μg/dl, n = 6; P < 0.001). Thereafter, cortisol levels returned to baseline values at 24 h (49 ± 3 μg/dl, n = 13). The serum level of cortisol remained at baseline values in animals treated with normal saline.

Expression of GR in lungs and other tissues. To examine the effects of methylprednisolone treatment and sepsis on protein expression of GRα, an isotype mediating the hormone action, the expression levels of GRα protein in lung tissues were evaluated by Western blotting using an antibody specific for GRα isoform. As presented in Fig. 1A, treatment of guinea pigs with 40 mg/kg methylprednisolone showed a significant decrease in total GRα protein expression in lungs. When sepsis was induced by intraperitoneal injection of 300 μg/kg LPS, the total expression level of GRα was significantly decreased. A more marked reduction in total GRα protein expression was found in lungs from the animals treated with methylprednisolone coadministered with LPS. On the other hand, methylprednisolone treatment and sepsis induction each resulted in a significant increase in GRα protein levels in nuclear fractions obtained from lungs (Fig. 1B). To our surprise, however, the GRα level in the nuclear fraction was dramatically reduced when the corticosteroid was given together with LPS.

GRα mRNA expression levels in lung tissues were analyzed by semiquantitative RT-PCR (Fig. 1C). The mRNA level of GRα was evidently reduced by methylprednisolone treatment. Also, downregulation of GRα mRNA was observed in lungs after sepsis induction with LPS. When the effect of the corticosteroid coadministered with LPS was examined, pulmonary GRα mRNA showed a more striking reduction than with each treatment alone.

Immunofluorescent staining for GRα confirmed the results obtained by immunoblot analysis. Thus immunoreactivity for GRα was less abundant in lung tissues from guinea pigs after
sepsis induction with LPS or after treatment with methylprednisolone compared with that observed in control lung tissues. Furthermore, when methylprednisolone was given together with LPS challenge, strongly reduced immunofluorescent staining for GRα was observed in guinea pig lung tissues (Fig. 2).

The relative expression of GRα protein in several control tissues is shown in Fig. 3A. GRα was highly expressed in lungs. Kidney and spleen expressed about 50–70% of the level in lungs, whereas the expression level of GRα in heart was only 37% and in liver was less than 10% that of lungs. In both kidney and spleen, after methylprednisolone treatment or LPS challenge, GRα protein levels were decreased, and this decrease was more marked when the corticosteroid was given together with LPS (Fig. 3B), which essentially corresponds with the results obtained in lungs.

We also compared pulmonary expression levels of GRβ, which does not bind glucocorticosteroids and is transcriptionally inactive (3, 40, 41), after methylprednisolone treatment and sepsis induction. As presented in Fig. 4, Western blotting results showed that the total expression level of GRβ protein was greatly reduced in lungs from guinea pigs treated with methylprednisolone compared with the control group. In contrast, sepsis induction with LPS resulted in a significant increase in total GRβ protein expression in lungs. This sepsis-induced increase in total GRβ protein expression was significantly but incompletely inhibited by methylprednisolone treatment. GRβ was undetectable in the nuclear fraction regardless of whether corticosteroid or LPS was administered.

In an attempt to identify the localization of GRα in the lung, immunofluorescent labeling techniques were conducted. Double labeling of normal guinea pig lung tissues using GR antibody with the type I and type II cell marker, cytokeratin, or the type II cell marker, SP-C, showed that neither type I nor type II cells were the major cells to express GRα in alveoli (Fig. 5, A and B). In addition, colocalization of GRα with the vascular endothelial cell marker, vWF, was not observed (Fig. 5C). Furthermore, lung sections from normal animals were triple labeled for macrophages and vascular smooth muscle cells with GRα, using specific markers, CD68 and αSMA. Triple labeling indicates that macrophages and vascular smooth muscle cells do not stain for GRα (Fig. 5D).

**Effect of methylprednisolone on septic lung inflammation.**
To examine the activation of NF-κB in guinea pig lungs, we measured NF-κB translocation to the nucleus with the antibody to an NF-κB subunit of p65. As depicted in Fig. 6A, translocation of NF-κB to the nucleus was markedly increased in lungs from guinea pigs after sepsis induction with LPS. Methylprednisolone effectively abolished this sepsis-triggered NF-κB nuclear translocation. Furthermore, analysis of NF-κB binding activity was performed in nuclear extracts of lungs by gel mobility shift assays (Fig. 6B). LPS challenge led to an increase in NF-κB binding activity. This induction of NF-κB binding activity by LPS was effectively eliminated when methylprednisolone was given to the animals. The addition of excess unlabeled NF-κB oligonucleotides competed with labeled NF-κB probe, demonstrating the specificity of the protein/DNA interaction (Fig. 6B, lane 6).

As shown in Fig. 7, quantitation of inflammatory cells detected in BALF represented a significant increase in the animals after sepsis induction with LPS compared with the control group. The sepsis-induced increase in BALF cell counts was blunted by treatment with methylprednisolone. Staining for differential counting showed the lavage cells contained >60% macrophages in all studies. This proportion remained substantially unchanged by sepsis induction and corticosteroid therapy. Thus many of inflammatory cells that displayed an evident increase in numbers after sepsis induction were macrophages.

**Effect of methylprednisolone on sepsis-induced cell apoptosis.** As depicted in Fig. 8, immunofluorescent staining for the cleaved form of caspase-3, a member of the caspase superfamily that initiates cell apoptotic events, showed that its protein expression was clearly detectable in lungs from methylprednisolone-treated or LPS-challenged guinea pigs but not in control lungs. Methylprednisolone, administered simultaneously with LPS, resulted in a much greater increase in activated caspase-3 in lungs compared with each treatment alone.
To assess whether sepsis and corticosteroid therapy can modify apoptotic cell death, the tissues were labeled with an in situ TUNEL assay. As shown in Figs. 9 and 10, TUNEL-positive apoptotic cells in lungs were evidently increased when sepsis was induced with LPS. In guinea pigs treated with methylprednisolone coadministered with LPS, TUNEL-positive cells had a tendency to further increase. This was more striking in spleen tissues. When the corticosteroid was given together with LPS, a marked increase in TUNEL-positive blood cells was observed. Significant increases in TUNEL-positive apoptotic cells in lung and spleen tissues were found when guinea pigs were given methylprednisolone alone, although this effect tended to be less pronounced compared with its administration to the LPS-treated animals. Treatment with low-dose hydrocortisone (5 mg·kg\(^{-1}\)·day\(^{-1}\) ip, 7 days) did not cause any increase in apoptotic cells in either lungs or spleen of guinea pigs [TUNEL-positive cells: 0.38 ± 0.04 for lung; 2.05 ± 0.31 for spleen (compare with controls in Fig. 10)].

**DISCUSSION**

Glucocorticoids have been the first, among anti-inflammatory agents, to be tested in large randomized controlled trials in septic shock, but their use is still controversial. In the Annane et al. (2) trial, it has been suggested that corticosteroid therapy reduces the morbidity effect of septic shock and may favorably affect survival from sepsis. On the other hand, several randomized controlled trials have shown unequivocally no value of high-dose glucocorticoid administration in patients with septic shock and ARDS (27, 44, 51, 59). In the present study, methylprednisolone treatment of guinea pigs was performed at a high dose (40 mg/kg ip). We found that high-dose methylprednisolone decreased expression of GR\(_{\alpha}\) in guinea pig lungs. Furthermore, sepsis, induced by LPS, resulted in a significant decrease in pulmonary expression of GR\(_{\alpha}\). Interestingly, when the corticosteroid was given together with LPS, the GR\(_{\alpha}\) expression level in lungs was reduced much more than with each treatment alone. There was a significant correlation between decreases in GR\(_{\alpha}\) protein and mRNA levels, as determined by RT-PCR analysis. This parallel behavior of protein and mRNA expression implies that the expression level of GR\(_{\alpha}\) in lung tissues is regulated in a transcriptional manner by both sepsis induction and corticosteroid therapy.

In accordance with previous studies showing that plasma levels of glucocorticoids are elevated during sepsis (21, 22), we found a marked increase in serum cortisol levels in guinea pigs treated with LPS in a time-dependent fashion. Increased glucocorticoids would be generally associated with downregulation of expression and ligand binding activity of GRs (11, 15, 43, 47, 55). Our observations that GR\(_{\alpha}\) expression was significantly decreased in lungs as well as kidney and spleen from LPS-induced septic guinea pigs are in close agreement with the results of other investigators showing that sepsis can downregulate GR numbers in different cell types and tissues (29, 52, 53). Furthermore, LPS infusion in pigs has been shown to downregulate GR expression in systemic organs, including lungs (16). Thus endotoxemia and/or sepsis appear to result in a general reduction in systemic GR\(_{\alpha}\) expression levels. In another study, however, the production and amount of GRs have been documented showing an increase in skeletal muscle during sepsis (54). The reasons for such unexpected results may be related to differences in animal age and species, septic models, and/or tissues used in that study, but the apparent discrepancy could be explained by the possibility that the increased number of GRs was reflective of an increase in GR\(_{\beta}\) levels being included.

**Fig. 7.** Effect of treatment with GC (40 mg/kg ip) on bronchoalveolar lavage fluid (BALF) cells in control and LPS-challenged guinea pigs. After BALF was collected from lungs, total inflammatory cells were counted with a hemocytometer. Values are means ± SE; n = 4. *P < 0.05 vs. control.

**Fig. 8.** Immunofluorescent images for cleaved caspase-3 (green) in lung tissues from control and LPS-challenged guinea pigs with and without GC treatment. In the bottom panels, nuclei were stained with Hoechst (blue). No apparent difference in fluorescent signals from nuclei among groups was noted. Representative images from 2 separate experiments are shown.
In contrast to GRα, GRβ interacts weakly with heat shock protein, does not bind glucocorticoids, and is transcriptionally inactive (12, 18). Various studies have shown that GRβ exerts a dominant-negative effect on GRα transcription of target genes, which may involve competition between GRα and GRβ for binding to GREs, competition for coactivators in the nucleus, as well as formation of GRα-GRβ heterodimers, which are transcriptionally inactive (3, 14, 58). Our study demonstrated for the first time that GRβ expression in lungs was upregulated by sepsis induction with LPS. Although methylprednisolone treatment resulted in a marked reduction in GRβ expression levels, sepsis-induced GRβ upregulation was still observed substantially even when the corticosterone was given. Increased expression of GRβ has been found in several diseases, which are associated with insensitivity to glucocorticoids, suggesting that an increased ratio of GRβ-to-GRα may induce glucocorticoid insensitivity (19, 23, 24, 28). The mechanism underlying the considerable increase in GRβ expression by sepsis induction is not fully understood, but this change may be important because increased GRβ in the cytoplasm could interfere with nuclear translocation of GRα, expression of which is strikingly reduced by sepsis with methylprednisolone treatment, in response to the corticosteroid, plausibly as a result of heterodimer formation between GRα and GRβ, leading to lower glucocorticoid sensitivity. Indeed, in lung tissues from septic guinea pigs receiving high-dose methylprednisolone, nuclear translocation of GRα was much less pronounced despite our observation that the corticosteroid can induce GRα nuclear translocation. It should be noted, however, that the elevated intrinsic glucocorticoid levels played a significant role in increased GRα nuclear translocation during sepsis without any corticosteroid therapy.

In guinea pigs, high expression of GRα was observed in lungs. The localization and distribution of GRs at mRNA and protein levels have also been demonstrated in human lungs by means of in situ hybridization and immunohistochemistry (1). Our immunofluorescent labeling study indicated that there was little expression of GRβ observed in type I and type II cells,
vascular endothelial and smooth muscle cells, and CD68-positive macrophages of guinea pig lung tissues. Thus we did not clarify well the localization of GR in macrophages are highly susceptible to LPS-induced lethality (6). To our surprise, however, high-dose methylprednisolone treatment strongly blocked sepsis-induced NF-κB activation, as indicated by a significant elimination of increases in nuclear translocation and binding activity of NF-κB. NF-κB is a proinflammatory transcription factor, and its activation is a central molecular event leading to the development of septic shock and inflammation (30). In the present study, LPS-induced sepsis resulted in a significant increase in the number of inflammatory cells from the BALF of guinea pigs, which was blunted by high-dose methylprednisolone treatment. Since nuclear translocation of GR affects the anti-inflammatory actions of the corticosteroid.

Recent accumulating evidence suggests that apoptosis plays an important role in the pathophysiology of sepsis, and apoptosis may be potentially detrimental in septic acute lung injury. Studies with the use of transgenic and gene knockout mice, providing protection against apoptotic cell death, have shown that these animals can exhibit a significant survival benefit in polymicrobial sepsis (13, 26). Moreover, we (37) and others (57) have demonstrated that silencing of gene transcripts necessary for apoptosis, such as Fas and caspase-8/caspase-3 via RNA interference, significantly improves the survival of septic mice. Glucocorticoids are known to induce apoptosis through mitochondria-derived caspase-9 activation of caspase-3 (42). In the present study, immunofluorescent staining for activated caspase-3 was enhanced, and the appearance of TUNEL-positive cells was further increased in septic guinea pig tissues treated with high-dose methylprednisolone. This shows that the increased process of cell apoptosis that occurs during sepsis can be significantly aggravated when glucocorticoid treatment is performed at high doses. In particular, extensive apoptosis of blood monocytes, including lymphocytes, would bear the risk of aggravated immunosuppression and increased incidence of secondary infections. Severe immunosuppression is one of the clinically important adverse effects accompanying corticosteroid therapy in septic shock. We thus suggest that glucocorticoid-induced cell apoptosis may be one of the mechanisms by which high-dose corticosteroid therapy can display a detrimental effect on septic mortality.

In conclusion, we showed that high-dose methylprednisolone administration in LPS-induced septic guinea pig lungs resulted in remarkable changes in expression of GRs in lungs, downregulated GRα, and upregulated GRβ, leading to impaired GRα nuclear translocation. Nevertheless, the ability of the corticosteroid to mitigate sepsis-induced NF-κB activation and lung inflammation was evidently preserved. Sepsis induction led to a significant increase in cell apoptosis, which was apparently enhanced by glucocorticoid treatment. Therefore, our present results suggest that the action of glucocorticoids to accelerate sepsis-induced cell apoptosis may overwhelm the therapeutic benefit of glucocorticoids in sepsis.

ACKNOWLEDGMENTS

We thank Megumi Matsui for excellent secretarial assistance. We are also grateful to Lesley D. Riley for proofreading.

REFERENCES


