Corticosterone Suppresses the Proliferation of RAW264.7 Macrophage Cells via Glucocorticoid, But Not Mineralocorticoid, Receptor

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Received November 4, 2012; accepted January 26, 2013; advance publication released online February 6, 2013

Macrophages are white blood cells within tissues that are produced by monocytes and help to protect against infection by bacteria through phagocytosis. Several studies have shown a correlation between the state of depression and abnormalities in the immune response. Corticosterone (CORT), which is often referred to as the stress hormone, is a well-known regulator of peripheral immune responses and also shows anti-inflammatory properties in the body. However, it is still unclear how CORT regulates macrophage function. In this study, we focused on the effects of CORT on the proliferation and survival of macrophage cells using the macrophage cell line RAW264.7. Under treatment with 10μM CORT for 24h, the proliferation of RAW264.7 cells decreased to 73.6% of that in the control. Moreover, this inhibition was blocked by treatment with mifepristone, a glucocorticoid receptor (GR) antagonist, but not by spironolactone, a mineralocorticoid receptor (MR) antagonist. In an lactate dehydrogenase (LDH) assay, CORT did not show any cytotoxic effect on RAW264.7 cells. JC-1 cell staining also showed that CORT did not influence mitochondrial dysfunction in RAW264.7 cells. In an investigation of the modulation of a signaling cascade by CORT, treatment with CORT promoted the translocation of GR, but not MR, from the cytosol to the nucleus in RAW264.7 cells. In conclusion, our findings suggest that CORT suppresses the proliferation of RAW264.7 cells by controlling the transcription of a particular gene, which is related to cell proliferation, through the formation of a CORT–GR complex.

Key words glucocorticoid; mineralocorticoid; corticosterone; RAW264.7; mifepristone; spironolactone

Corticosterone (CORT), which is classified as a steroid hormone, is an adrenal corticosteroid that plays a role in a wide range of phenomena, such as the regulation of glucose metabolism, the reaction of the immune system and the stress response. CORT is one of the main corticosteroids in rodents. There are two types of adrenal corticosteroid receptors: type II, or glucocorticoid receptor (GR), and type I, or mineralocorticoid receptor (MR).1,2 It has been demonstrated that CORT has greater affinity for MR than for GR.3,4 In general, both GR and MR are localized in the cytosol. It has been demonstrated that a ligand-steroid hormone receptor complex forms a homodimer together with another chaperon protein once CORT binds to steroid hormone receptor. After it forms a homodimer complex, this complex translocates from the cytosol to the nucleus and binds to a particular motif of DNA to regulate DNA transcription.5 In the case of GR, intracellular GR is inactivated by the formation of complexes with several proteins, such as heat-shock proteins.6,7 After the active ligand-steroid hormone receptor complex forms a homodimer and translocates to the nucleus from the cytosol, it modulates the transcriptional responses of inflammatory genes, either by binding directly to DNA or by cooperating with some other protein or signal cascade, such as nuclear factor κB (NFκB),8 Jun N-terminal kinase (JNK),9,10 p38 mitogen-activated protein kinase (p38 MAPK)9 or cAMP response element-binding protein (CREB).11 Consequently, CORT can show an anti-inflammatory effect by modulating the production of a cytokine, such as tumor necrosis factor-α (TNF-α) or interleukin (IL-1β).12

Under stressful conditions, which include both physical and mental stress, the concentration of CORT in both blood and tissue increases. Although CORT has a potent anti-inflammatory effect, many studies have shown that CORT also has an inhibitory effect on cell proliferation and a cytotoxic effect toward several types of cells. Numerous studies have indicated that prolonged and excessive secretion of CORT damages brain neurons and modifies their network. The chronic secretion of CORT induced by stress increases the incidence of hippocampal neuron loss in rats.13 In addition, it has been reported that cell proliferation in the hippocampus also decreased under chronic treatment with low-dose CORT in vitro and chronic stress stimulation in vivo.14–16 Moreover, it has been shown that the proliferation of not only neurons but also astrocytes is inhibited in the cortex.17 These findings suggest that CORT, which is secreted under stressful conditions, regulates the proliferation and survival of different types of cells in the brain and tissues.

Macrophages are white blood cells within tissue that help to protect against infection by bacteria through phagocytosis. Several studies have shown a correlation between the state of depression and abnormalities in the immune response, such as increased numbers of white blood cells, alterations in sub-populations of leucocytes, suppression of the cytotoxic activity of natural-killer cells, and changes in the levels of some autoantibodies and cytokines. It has been demonstrated that mice that were exposed cold stress showed reduced numbers of thymocytes and splenocytes, decreased T-cell blastogenesis, and reduced natural-killer cell activity.18 Moreover, it has been reported that chronic cold stress induced a regulatory phenotype in macrophages, characterized by diminished phagocytic ability, decreased TNF-α and IL-6, and increased IL-10 production.19

The authors declare no conflict of interest.

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These findings suggested that the functions of macrophage cells are regulated by CORT, which is secreted under stressful conditions. In this study, we investigated the effects of CORT on the proliferation and survival of macrophage cells using the cell line RAW264.7, as well as the underlying molecular mechanisms.

MATERIALS AND METHODS

Cell Culture RAW264.7 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) with high glucose (Wako Pure Chemical Industries, Ltd., Osaka, Japan) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin in a humidified incubator under a 95%/5% mixture of air and CO₂. Cells were generally passaged every 5 d.

WST-8 Assay RAW264.7 cell proliferation was analyzed by a WST-8 assay using a Cell Counting Kit-8 (Dojindo, Kumamoto, Japan). Once cells became confluent, they were plated into 96-well microplates at a density of 5×10³/mL (5×10⁴/well) with CORT and antagonists. CORT and the antagonists mifepristone and spironolactone (Sigma-Aldrich, St. Louis, MO, U.S.A.) were dissolved in dimethyl sulfoxide (DMSO). When cells were treated with CORT and antagonists, the final concentration of DMSO was set at 0.2% with respect to the cell culture medium. After 24, 48 and 72 h of culture, the cells were incubated with WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium] solution at 37°C for 2 h. The spectrophotometric absorbance of WST-8/formazan produced by dehydrogenase activity in living cells was measured at a wavelength of 450 nm using a VersaMax (Molecular Device, Tokyo, Japan). Absorbance at 650 nm was also measured as a reference. The spectrophotometric absorbance measured by this assay is highly correlated with the number of living cells.

Lactate Dehydrogenase (LDH) Assay The cytotoxicity of CORT against RAW264.7 cells was analyzed by an LDH assay using an LDH Cytotoxicity Detection Kit (TaKaRa, Otsu, Japan). Cells were plated into 96-well microplates at a density of 1×10⁵/mL (5×10⁶/well) with CORT and antagonists. In this experiment, cells were cultured with only 1% FBS culture medium because LDH that is contained naturally in FBS induces a high background signal for analysis. Cells were also cultured in culture medium containing 1% NP-40 (MP Biomedicals, Solon, OH, U.S.A.) to determine the maximum LDH activity from cells under maximum cytotoxicity. After 24 h, supernatant of the culture medium was collected by centrifugation and incubated with reagents at room temperature for 30 min. The spectrophotometric absorbance of 2-[4-indophenyl]-3-[4-nitrophenyl]-5-phenyltetrazolium chloride produced by lactate dehydrogenase activity that was released from damaged cells was measured at a wavelength of 492 nm using a VersaMax. Absorbance at a wavelength of 600 nm was also measured as a reference as mentioned above. The spectrophotometric absorbance measured by this assay is highly correlated with the number of dead cells.

Immunocytochemistry Cells were plated onto slide glass (Matsunami Glass Ind., Osaka, Japan) that had been coated with poly-l-lysine (Sigma-Aldrich, St. Louis, MO, U.S.A.). After 5 d of culture, cells were fixed with 4% paraformaldehyde in phosphate-buffered saline. After the addition of 3% FBS containing phosphate-buffered saline for blocking, cells were permeabilized with 0.1% Triton X-100 in phosphate-buffered saline to stain intracellular proteins. Cells were then incubated with anti-GR polyclonal antibody (Santa Cruz, 1:250) and anti-MR polyclonal antibody (Santa Cruz, 1:250). Alexa Fluor 546-conjugated anti-rabbit IgG antibody was used as a secondary antibody. Stained cells were observed and photographed under an Olympus DP70 fluorescence microscope equipped with a digital camera and a DP70 controller.

JC-1 Staining The dye JC-1 (5,5,6,6-tetrachloro-1,1,3,3-tetraethylbenzimidazolylcarbocyanine iodide; Invitrogen, Carlsbad, CA, U.S.A.) has the unique characteristic of being accumulated in mitochondria that have a high membrane potential in living cells. Moreover, the color of this dye changes reversibly from green to orange as the mitochondrial membrane potential increases. To analyze the effect of CORT on the mitochondrial membrane potential, RAW264.7 cells were stained with JC-1. After cells were plated onto slide glass coated with poly-l-lysine and cultured for 24 h with CORT and antagonist, they were then incubated with 10 μM JC-1 at 37°C for 30 min. The cells were then washed to remove extra dye. Stained cells were observed and photographed with an Olympus DP70 fluorescence microscope equipped with a digital device camera controlled by a DP70 controller.

Western Blotting Protein samples were obtained from RAW264.7 macrophage cells that had been exposed to 10 nM CORT for 1, 3, 6, 24 and 72 h. To prepare protein extract from RAW264.7 macrophage cells, cells were washed with ice-cold homogenizing buffer (250 mM sucrose, 2 mM ethylene-diaminetetraacetic acid (EDTA) 2Na, 10 mM ethylene glycol tetraacetic acid (EGTA) and 20 mM Tris–HCl; pH 7.4) containing a protease inhibitor cocktail (Roche, Basel, Switzerland) and centrifuged to obtain a cell pellet. The cell pellet was treated with lysis buffer (homogenizing buffer containing 1% Triton X-100) and sonicated on ice. After the sample was centrifuged at 10 000×g for 30 min at 4°C, the supernatant was collected as a total protein extract. To prepare the cytosol protein extract, the cell pellet was incubated for 30 min on ice with lysis buffer (homogenizing buffer containing 1% Triton X-100). After the supernatant was centrifuged, the supernatant was collected as the cytosol protein extract. After the supernatant was collected, lysis buffer was again added to the residue of the pellet and the sample was sonicated to completely dissolve into lysis buffer. After centrifugation, the supernatant was collected as nuclear protein extract. Protein concentrations were determined by using a bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, U.S.A.) and protein extracts were boiled with equal volumes of 2× sodium dodecyl sulfate (SDS) sample buffer (BioRad, Hercules, CA, U.S.A.). For Western blotting, protein extracts were placed on 8% SDS-polyacrylamide gel, transferred to a polyvinylidene difluoride membrane (BioRad) and immunoblotted with anti-GR polyclonal antibody (Santa Cruz, M-20, 1:2000), anti-MR polyclonal antibody (Santa Cruz, H-300, 1:2000), anti-c-Fos polyclonal antibody (Santa Cruz, 1:2000), anti-p38 MAPK polyclonal antibody (Cell Signaling, 1:1000), anti-phosphor-p38 polyclonal antibody (Cell Signaling, 1:1000), anti-CREB monoclonal antibody (Cell Signaling, 1:1000), anti-phospho CREB monoclonal antibody (Cell Signaling, 1:1000), anti-p44/42 MAPK polyclonal antibody (Cell Signaling, 1:1000), anti-phospho-p44/42 MAPK polyclonal antibody (Cell Signaling, 1:1000).
ing, 1:1000), anti-stress-activated protein kinase (SAPK)/JNK polyclonal antibody (Cell Signaling, 1:1000), anti-phospho-SAPK/JNK polyclonal antibody (Cell Signaling, 1:1000), anti-NFκB p65 subunit monoclonal antibody (Chemicon, 1:2000), anti-inhibitor of IκB monoclonal antibody (Santa Cruz, 1:1000) and anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) monoclonal antibody (Millipore, 1:5000). The blots were developed with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG (Jackson ImmunoResearch, 1:5000) and visualized by chemiluminescence using an ECL Plus Western Blotting Detection System (Amersham, Piscataway, NJ, U.S.A.). The blotting image was obtained and digitalized using by ChemiDoc™ XRS+ systems (BioRad). The intensity of each image was analyzed using by Quantity One 1-D Analysis Software (BioRad).

Statistics The results are presented as the mean±S.D. A statistical analysis was performed using the Dunnett multiple comparisons test. A p value < 0.05 was considered to be statistically significant.

RESULTS

Inhibitory Effect of CORT on the Proliferation of RAW264.7 Cells First, we investigated whether or not

![Images of RAW264.7 cells cultured with and without 10 µM CORT (A). Pictures were taken after treatment with CORT for 24 h. Scale bar represents 200 µm (upper) and 50 µm (lower), respectively. WST-8 cell proliferation assay of RAW264.7 cells cultured with and without CORT (B). The vertical axis shows the actual absorbance at 450 nm. The histogram shows means±S.D. (n=12, *p<0.05 vs. control). WST-8 cell proliferation assay of RAW264.7 cells cultured with and without CORT (C). The vertical axis shows the proliferation rate relative to that in the control. DMSO was used as a vehicle to prepare CORT solution. The histogram shows means±S.D. (n=12, *p<0.05 vs. vehicle).]
CORT affected the proliferation of RAW264.7 cells. As shown in Fig. 1A, the proliferation of RAW264.7 cells was decreased by treatment with 10 µM CORT for 24 h compared with the control in low-magnification images. At high magnification, no changes in the morphology of RAW264.7 cells were observed under treatment with 10 µM CORT. Next, we performed a WST-8 assay to evaluate the effect of CORT on the proliferation of RAW264.7 cells (Figs. 1B, C). Figure 1B shows the actual absorbance in the WST-8 assay. CORT at 10 µM inhibited the proliferation of RAW264.7 cells at 24, 48 and 72 h of incubation compared with the control. Figure 1C shows the cell proliferation under each culture condition as a percentage of that in the control. Under treatment with CORT, the proliferation of RAW264.7 cells was significantly decreased to less than 80% of that in the control within the initial 24 h of incubation, and this decrease persisted for at least 72 h. Moreover, this inhibitory effect on the proliferation of RAW264.7 cells was dose-dependent. These results suggested that CORT has an inhibitory effect on the proliferation of RAW264.7 cells.

Expression of Glucocorticoid Receptor and Mineralocorticoid Receptor in RAW264.7 Cells
To confirm whether both GR and MR are expressed in RAW264.7 cells, we performed immunocytochemistry and Western blotting analyses. Figure 2A shows the results of immunocytochemical staining with anti-GR and MR polyclonal antibodies. Both GR and MR antibody showed multiple bands, however, predicted molecular weight of GR is 95 kDa, on the other hand, that of MR is 102 kDa. RAW264.7 cells were stained with both anti-GR and anti-MR polyclonal antibody at the same level of intensity. This result is consistent with the result of Western blotting, which demonstrated the expression of both GR (approximately 90–95 kDa) and MR (approximately 102 kDa) in RAW264.7 cells (Fig. 2B).

Effects of Mifepristone and Spironolactone on the Proliferation of RAW264.7 Cells Induced by Corticosterone
To determine which receptor plays a role in the inhibitory effect of CORT on the proliferation of RAW264.7 cells, antagonist competition experiments were performed using mifepristone, a GR antagonist, and spironolactone, a MR antagonist. First, we identified the appropriate concentrations of both antagonists for this experiment. As shown in Figs. 3A and B, treatment with 0.1, 0.5 and 1 µM mifepristone, and 0.5, 2.5, and 5 µM spironolactone did not have any effect on the proliferation of RAW264.7 cells after 72 h. However, treatment with 25 and 50 µM spironolactone, and 5 and 10 µM mifepristone decreased the proliferation of RAW264.7 cells to 40% and 76% compared to that in the control, respectively. These results suggested that high concentrations of both antagonists, particularly spironolactone, had cytotoxic effects on RAW264.7 cells. In contrast, in the antagonist competition experiments, treatment with mifepristone at 0.5 µM and 1 µM, but not 0.1 µM, partially abolished the inhibitory effect of 10 µM CORT on RAW264.7 cell proliferation (Fig. 3A). On the other hand, treatment with spironolactone at all concentrations except for 50 µM did not influence the inhibitory effect of 10 µM CORT on RAW264.7 cell proliferation (Fig. 3B).

Cytotoxic Effect of Corticosterone on RAW264.7 Cells
To confirm whether the inhibitory effect of CORT on the proliferation of RAW264.7 cells was due to a cytotoxic effect, an LDH assay was performed. Figure 4 shows the results of an LDH assay for RAW264.7 cells that were treated with CORT. Under the control culture conditions, the percentage of dead cells, where the value obtained under treatment with 1% NP-40 was taken to represent 100% cell death, was 10%. The absorbance under the control culture conditions might be due to the use of serum-reduced medium in this experiment. In comparison with the control culture condition, treatment with vehicle or 0.1, 1 and 10 µM CORT did not show any significant difference in the cytotoxic effect on RAW264.7 cells.

Effect of Corticosterone on the Mitochondrial Membrane Potential in RAW264.7 Cells
Several studies have reported that CORT affects mitochondrial function including energy metabolism.20,21 These reports inspired us to investigate mitochondrial function in RAW264.7 cells under treatment with CORT. In general, high mitochondria membrane potential indicates the healthy energy metabolism condition of mitochondria. In this experiment, we examined the mitochondrial membrane potential in living RAW264.7 cells by using the mitochondrial probe JC-1. Figure 5 shows the results of JC-1 staining under various culture conditions. In both the control and vehicle-treatment conditions, orange fluorescence, which indicates a high mitochondrial membrane potential, was observed in most of the cells. Under treatment with 10 µM CORT, there was no difference in JC-1 staining compared to both the control and vehicle-treatment conditions. In addition, treatment with both 10 µM CORT and 1 µM mifepristone also did not produce any difference in the mitochondrial membrane potential compared with either the control or vehicle-treatment conditions. These data suggested that CORT did not effect to mitochondrial function.
Western Blotting of the Expression of Proteins Related to a Corticosteroid Signaling Cascade

First, we performed a Western blotting analysis to check the change of expression levels of GR and MR in the cytosol and nuclear fractions. In the cytosol fraction, the expression level of GR showed little, if any, change with an increase in the duration of exposure to CORT. On the other hand, the expression level of GR in the nuclear fraction increased by more than 180% upon treatment with CORT, even for only 1h (Fig. 6A). Surprisingly, treatment with 1 µm mifepristone, which is an antagonist for GR, also induced an increase in the level of GR expression in the nuclear fraction. In contrast, the expression levels of MR in both the cytosol and nucleus were not affected by treatment with CORT (Fig. 6B).

We expected that inactivation of NF-κB by GR-CORT complex might exhibit cell proliferation inhibitory effect. However, the expression levels of NF-κB and IκB, which is a protein that inhibits the function of NF-κB in
DISCUSSION

In this study, we investigated the effect of CORT, which is secreted in response to stress stimuli, on macrophages using RAW264.7 cells. We demonstrated that both GR and MR were expressed in RAW264.7 cells and that the proliferation of RAW264.7 cells was inhibited by treatment with CORT via GR, but not MR. Moreover, our observations also suggested that this inhibitory effect on the proliferation of RAW264.7 cells might be induced by translocation of a ligand–steroid hormone receptor complex directly from the cytosol to the nucleus.

Several studies have indicated that stress can suppress the immune response in most animals, including humans. It has been reported that mice that were exposed to cold water stress for 4d showed reduced numbers of thymocytes and splenocytes, decreased T-cell blastogenesis, and reduced NK activity. With regard to these phenomena, glucocorticoids, the concentrations of which increase in response to stress stimuli, mediate the surface phenotypes of peritoneal cells of the monocyte/macrophage lineage to change the polarity of macrophage cells. Moreover, in resting macrophages, corticosterone decreased phagocytosis mediated by Fc-gamma and mannose and beta-glucan receptors, while catecholamines had no effect. There is also direct evidence that rat macrophage cells, which were pretreated with CORT, reduced the production of TNF-α by the treatment with lipopolysaccharides. These findings suggest that glucocorticoids released in response to stress stimuli suppress the immune response by directly changing the activity and phenotype of macrophage cells. However, several studies have implied that there is a difference between the effects of physiological stress and social stress on the immune response. In particular, social disruption has been shown to increase cytokine production by monocytes/macrophages and to reduce their sensitivity to CORT and glucocorticoid resistance in mouse splenic macrophages. These discrepancies may be the result of complex interactions between hormones, cytokines, and particular phenotypes of cells and tissues in response to each stress stimuli.

Corticosteroid receptors, both GR and MR, are expressed in several immune cells and tissues. The localization of each receptor in immune cells and tissues is distinct, and reflects their specific role in the regulation of the immune system. It has been reported that macrophage cells possess several types of nuclear receptors and endocrine receptors, including GR and MR. However, it is still unclear how glucocorticoids regulate the function of macrophages. A recent study showed that a low concentration of CORT enhances the immune functions of macrophage cells, whereas a high concentration of corticosterone has immunosuppressive effects on macrophage cells. Lim et al. demonstrated that the nitric oxide production and mRNA expression induced by several cytokines are regulated depending on the concentration of glucocorticoids, and are mediated via GR, but not MR. As noted above, these trends are seen in not only macrophage cells, but also other types of cells. For example, GR and MR mediated opposite effects of CORT on the functions of microglial cells. Moreover, it has been reported that glucocorticoids have biphasic effects on mitochondrial function in cortical neurons via GR. In contrast, in an RNA interference experiment, knockdown of GR expression promoted the proliferation of RAW264.7 cells with a decrease in p27 expression and an increase in Protein kinase C-alpha expression. This result is consistent with our finding that the proliferation of RAW264.7 cells was inhibited by treatment with CORT and this inhibitory effect on the steady state, in both the cytosol and nuclear fractions also showed no changes (Fig. 7).
Fig. 6. The Expression of GR and MR in Cytosol and Nuclear Fractions of RAW264.7 Cells

The protein expression of GR in cytosol and nuclear fractions of RAW264.7 macrophage cells (A). The histogram shows means±S.D. (n=3–4, *p<0.05 vs. control). The relative expressions of both GR and MR were calculated with respect to the value of the control as 100%. Protein extracts of both cytosol and nuclear fractions were collected from cells cultured with 10 μM CORT for 1, 3, 6, 24, and 72 h. Otherwise, the protein extracts were obtained after 72 h of culture under each culture condition.
cell proliferation was completely abolished by treatment with mifepristone, a GR antagonist, but not spironolactone, a MR antagonist. CORT has also been shown to mediate the proliferation of other types of cells via GR. It has been reported that microglia isolated from the forebrain of newborn showed a change in morphology under in vitro treatment with CORT via GR. We also demonstrated that the proliferation of microglia cell line, BV-2, was suppressed with the treatment of corticosterone and this effect was antagonized with the treatment of mifepristone, but not spironolactone. In vivo studies have also shown that CORT interferes with the proliferation of various cell types in the brain. Moreover, the proliferation of microglia is mediated by various inflammatory cytokines, such as granulocyte macrophage colony-stimulating factor, interleukin-1 and tumor necrosis factor. These results support the notion that GR, but not MR, play a key role in cell proliferation and functional regulatory mechanisms of macrophage cells in the immune response.

When the expression levels of both GR and MR were investigated, the expression level of GR in the nuclear fraction was clearly increased after treatment with CORT. This result suggests that CORT-GR complex may translocate to the nucleus. It has been reported that dexamethasone, potent GR agonist, also induced translocation of GR, accompanied with its phosphorylation, from cytosol to nucleus in NIH-3T3 cells. Moreover, both GR phosphorylation level and its site are critical for regulation of transcription activity of GR which translocates to nucleus. In addition, it has been reported that GR regulates various gene transcription. Transcription activity of some genes related to cell cycle, such as cyclin, might be altered by CORT treatment. In contrast, the expression level of MR did not change in either the cytosol or nuclear fraction. This observation is consistent with the finding that the MR antagonist spironolactone did not abolish the

![Western blot analysis](image-url)
inhibitory effect of CORT on cell proliferation. These results suggest that translocation of GR induced by CORT treatment may be involved in the inhibitory effect of cell proliferation in RAW264.7 cells. Surprisingly, it has been observed that mifepristone, antagonist of GR, also could induce GR translocation though mifepristone did not show any effects on cell proliferation. However, it has been reported that mifepristone also have a potential as a partial agonist against GR for induction of GR translocation. This phenomenon indicates that the antagonistic effect of mifepristone against cell proliferation inhibitory effect induced by CORT might not be caused by inhibition of GR translocation. Otherwise, there is a possibility that mifepristone may inhibit phosphoryl-ated GR translocation from cytosol to nucleus. Further experiment would be needed to clarify the mechanisms of cell proliferation inhibitory effect of CORT via GR. When we checked the expression level of other proteins related to GR signaling cascades, the expression of c-fos, p44/42 MAPK and its phosphorylated forms did not change following treatment with CORT in both the cytosol and nuclear fractions. The expression levels of p38 MAPK and JNK, which are related to apoptosis, and their phosphorylated forms did not change. The expression of CREB and its phosphorylated form also did not change at all. Unexpectedly, there were no changes in the expression levels of NfκB and IκB in both the cytosol and nuclear fractions although the NfκB signaling pathway is known to be one of the major pathways for the GR signaling cascade. These results also support that GR play a critical role to the cell proliferation inhibitory effect by CORT in RAW264.7 cells.

In summary, CORT inhibited the proliferation of RAW264.7 cells. Moreover, this effect is involved with GR, but not MR, and may result in the alteration of transcription activity induced by CORT-GR complex. These results suggest that changes in the characteristics of macrophage cells induced by corticosteroids, which are secreted under conditions of acute or chronic stress, may play a role in suppression of the immune response. Further studies are needed to clarify the correlation between the function and the proliferative inhibition on macrophage cells under treatment with glucocorticoids in the immune response.

Acknowledgements We thank the members of the Department of Pharmacology, School of Pharmacy, International University of Health and Welfare, for their helpful discussions throughout this study. This work was supported in part by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan and for Scientific Research (C), 22600014, 2010, by the Suzuki Memorial Foundation and by an Internal Research Grant from the International University of Health and Welfare.

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