Regulation of glucocorticoid sensitivity in thymocytes from burn-injured mice

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Submitted 10 July 2008; accepted in final form 5 November 2008

D’Elia M, Patenaude J, Bernier J. Regulation of glucocorticoid sensitivity in thymocytes from burn-injured mice. Am J Physiol Endocrinol Metab 296: E97–E104, 2009. First published November 11, 2008; doi:10.1152/ajpendo.90582.2008.—Glucocorticoids (GC) are important steroid hormones that regulate metabolism, development, and the immune system. GC are produced continuously, and maximal levels are reached following stress-related stimuli. Previous studies have demonstrated that increased GC production following thermal injury was responsible for thymic involution. Although GC are mainly synthesized by the adrenal glands, there is increasing evidence that GC may also be produced in nonadrenal tissues. The thymus was reported to express steroidogenic enzymes and to release GC. 11β-Hydroxysteroid dehydrogenase type I (11β-HSD1) is predominantly a reductase in cells and is essential for the local reactivation of GC. Here, we report that increased GC-induced apoptosis in thymocytes from burn-injured mice is related to increased glucocorticoid receptor (GR) expression and 11β-HSD1 expression in thymocytes at day 1 postburn injury. In vitro, thymocytes were able to convert 11-dehydrocorticosterone (DHC) to corticosterone (CORT), which induced their apoptosis, and this was pharmacologically inhibited by 18β-glycyrrhetinic acid, a specific 11β-HSD inhibitor. Moreover, 11β-HSD1 expression was confirmed in the 267S3 thymoma-derived cell line, and its activity was responsible for greater sensitivity of these cells to CORT-induced apoptosis. Finally, proinflammatory cytokines [tumor necrosis factor-α, interleukin (IL)-1β, and IL-6] increased thymocyte sensitivity to DHC-induced apoptosis through a mechanism involving 11β-HSD1. Overall, we have shown that burn injury induced 11β-HSD1 expression in thymocytes, which led to a greater sensitivity of these cells to CORT-induced apoptosis. Increased expression of 11β-HSD1 and GR may play a role in intra-thymic T cell development and can be major determinants of GC sensitivity after a trauma.

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Previous studies using 18β-glycyrrhetinic acid (GA), an inhibitor of 11β-HSD, suggested a role for 11β-HSD1 in immunity (17, 18). Recently, 11β-HSD1 expression has been demonstrated in immune cells. Monocyte differentiation into macrophage correlated with increased expression and activity of 11β-HSD1 (37). Furthermore, 11β-HSD1 expression could be induced by “type 2” anti-inflammatory cytokines interleukin (IL)-4 and IL-13. It was suggested that IL-4 accelerated macrophage differentiation rather than directly induced 11β-HSD1 activity (37). Recent findings have demonstrated a link between 11β-HSD1 expression and the increased clearance of apoptotic neutrophil by macrophages (16). 11β-HSD1-deficient mice showed a delay in the clearance of neutrophils undergoing apoptosis and progression of experimental peritonitis (16). Also, 11β-HSD1 expression has been described in mouse and human dendritic cells, as well as in mouse thymocytes, T and B lymphocytes (13, 42). Nuotio-Antar and colleagues (22) have shown that 11β-HSD1 expression in the thymus increases from late fetal development to reach maximal levels in the adult. In the thymus, 11β-HSD1 expression and activity was ~6–7% of the levels observed in the liver and restricted to CD3+ thymocytes and epithelial cells (22). Recently, Zhang et al. (42) established that both CD4+ and CD8+ T cells in spleen expressed 11β-HSD1 mRNA, protein, and enzymatic activity. Current findings based on 11β-HSD1 expression in thymocytes support a role of prereceptor metabolism in GC sensitivity inside the cell.

In this context, we now demonstrate for the first time that a high-stress condition modulates 11β-HSD1 expression in the thymus. Soon after a severe burn injury, GC-induced thymocyte depletion was associated with an increase of both GR density and 11β-HSD1. Using freshly isolated thymocytes and the thymoma cell line, we showed that the expression of 11β-HSD1 resulted in amplification of CORT sensitivity. Moreover, inflammatory cytokines potentiated this effect of amplification.

MATERIALS AND METHODS

Animals. All experiments were performed on 6-wk-old male mice: C57BL/6 (Charles River Laboratories, St. Constant, Quebec, Canada). The mice were acclimatized for a period of 2 wk before the initiation of any procedures and were housed in a central animal facility under strictly controlled temperature, relative humidity, and a 12:12-h light-dark cycle. They were kept in cages, each containing five mice. Standard chow (Richmond Standard Lab Diet; Lab Diet, Richmond, IN) and water were provided ad libitum. The Institutional Animal Care Committee reviewed and approved all procedures performed in accordance with the Canadian Council on Animal Care guidelines.

Burn injury. Mice were subjected to a 20% total body surface area burn as described previously (6). Briefly, mice were randomly assigned to a control or burn group, each containing eight animals. After anesthesia and shaving of the dorsum, animals were placed in a mold and immersed for 7 s in water at 90°C to produce a full thickness to cover 20%. The sham group was immersed in water at room temperature (22°C). Both sham- and burn-injured mice were resuscitated after the procedure with 2 ml of saline given intraperitoneally containing buprenorphine. Flavamine (Smith & Nephew, Montreal, Quebec, Canada), a topical antiinfective, was directly applied on the wound to reduce the risk of infection.

Cell preparation and cell line. On the day of the experiment, mice were killed, and the thymus was removed and washed. Thymocyte isolation was performed individually as single-cell suspensions by teasing thymus against a steel net in RPMI 1640 medium supplemented with 10% heat-inactivated FBS (Sigma-Aldrich) and antibiotic solution (100 IU/ml penicillin and 100 g/ml streptomycin; Sigma-Aldrich). Each eluted cell suspension was washed two times with RPMI 1640 and preserved at 4°C. TEC were obtained from extensively washed thymic stromal tissues (TST) obtained from the remaining part of the thymus on the net. TST were digested with a solution of collagenase D (1 mg/ml)DNase (20 μg/ml) (Roche Diagnostics, Laval, Quebec, Canada) in RPMI 1640 for 1 h at 37°C. Residual cells were washed three times and resuspended in RPMI. Cell viability was consistently >90%. Purity of each thymocyte suspension and TEC suspension was determined by flow cytometric analysis using anti-CD3 and anti-CD24 monoclonal antibodies for staining. In thymocyte populations, 97% were positive for one of either marker. In TEC suspension, ~95% of cells were negative for both markers. The mouse thymic lymphoma line 267S3 (CD3+ CD8+CD4+) was developed in vitro from a radiation-induced thymus lymphoma and isolated in the spleen after three rounds of injection in syngeneic mice (kindly provided by Dr. Yves St. Pierre, Institut National de la Recherche Scientifique-Institut Armand-Frappier). Western blot analysis. Western blot analysis of GR and 11β-HSD1 was performed for thymocytes and isolated TEC. Cells were lysed with RIPA buffer, proteins were dosed by Bradford, and 40 μg of protein were mixed in sample buffer, subjected to SDS-PAGE, and electroblotted on a nitrocellulose membrane (VWR, Montreal, Quebec, Canada). The membranes were washed in Tris-buffered saline (TBS) and blocked 3 h at 37°C in TBS containing 5% nonfat dry milk (Fisher Scientific) and 0.1% Tween 20 (Fisher Scientific). The membranes were then incubated overnight at 4°C with rabbit anti-mouse GR (M-20; Santa Cruz Biotechnology, Santa Cruz, CA) or rabbit anti-mouse 11β-HSD1 (Alpha Diagnostic International, San Antonio, TX). Specific antibody-antigen complexes were revealed using a horseradish peroxidase-labeled anti-rabbit antibody (SC-2004; Santa Cruz Biotechnology) and ECL Western blotting detection reagents (Amersham Biosciences). Membranes were stripped and probed with a mouse anti-actin (A4700; Sigma-Aldrich) as a loading control.

Semiquantitative PCR. Total RNA was isolated with TRizol reagent (Invitrogen, Burlington, Ontario, Canada) and was further treated with DNase I (Invitrogen) to remove potential contamination by genomic DNA, followed by reverse transcription. Aliquots were subjected to PCR amplification with Taq polymerase (Qiagen, Mississauga, Ontario, Canada). The following primers were used: mouse 11β-HSD1 forward primer: 5′-TGG AGC GCC AAC AAA AAG GAG; mouse 11β-HSD1 reverse primer: 5′-CCA GCA AGT TAG TGA CCA AGAG. Vascular cell adhesion molecule (VCAM)-1 primers and bEnd.3 endothelioma cell line RNA extract (VCAM-1 positive control) were kindly provided by Dr. Yves St-Pierre (35). The amount of RNA loaded for each sample was observed by the expression levels of gyceraldehyde-3-phosphate dehydrogenase mRNA.

DHC and CORT treatment. Briefly, 1 × 106 isolated thymocytes from sham- and burn-injured mice or 267S3 thymoma cell line were cultured in 24-well plates in complete RPMI supplemented with 10% FCS (Sigma-Aldrich) for 24 h and exposed to 1 μM DHC (Sigma-Aldrich) or CORT (10–6 to 10–8 M). In some experiments, cells were treated with 100 nM RU-486 (Sigma-Aldrich) for 30 min before CORT exposition. In other experiments, cells were pre-treated for 30 min with 10 μM of the 11β-HSD inhibitor GA (Sigma-Aldrich). When evaluating the effect of proinflammatory cytokines on DHC-induced apoptosis, cells were also exposed to 1–10 nM of murine tumor necrosis factor (TNF)-α or IL-1β or IL-6 (Preprotech, London, UK).

Apoptosis determination. T cell apoptosis was evaluated after staining with propidium iodide and by quantifying the sub-G0/G1 population in flow cytometric analysis as described previously (7). Following culture, cells were collected and washed two times with PBS. The pellet was then resuspended in 300 μl Krishan staining buffer. RNase (10 μg) was added, and the tubes were placed in the
dark at 37°C for 1 h. Apoptosis was quantified by flow cytometry on a FACScan flow cytometer (Becton-Dickinson) by measuring the percentage of cells in phase sub-G₀/G₁. Ten thousand cells were analyzed for each test.

Statistical analysis. All data are expressed as means ± SE. Data were analyzed by one-way ANOVA with the Tukey comparison test using the GraphPad InStat 3.05 software program (GraphPad Software, San Diego, CA). Differences between groups were considered statistically significant when the probability by chance was <5% (P < 0.05). All experiments were performed in quadruplicates or more when indicated, to confirm our results.

RESULTS

Thymocytes from burn-injured mice have increased GR expression and are more sensitive to CORT-induced apoptosis. In a previous study, we demonstrated that thymic involution at day 1 following burn injury was related to an increase in plasma free CORT (15). In the present work, we wanted to address GC sensitivity of thymocytes in burned mice. At day 1 following burn injury, thymocytes of both sham and burn groups were cultured in the presence of increasing CORT concentrations (0.01–1 μM). Results show that, at the optimal concentration of CORT (1 μM), thymocytes from both groups had the same sensitivity to CORT, as shown by the percentage of apoptotic cells (Fig. 1A). Interestingly, exposure to a moderate concentration of CORT (0.1 μM) resulted in a significant increase of apoptotic cells in the burn group compared with the control group of mice (Fig. 1, A and B). At a lower concentration (0.01 μM), no significant difference was observed between either group. In vitro increased sensitivity to CORT of thymocytes from burn-injured mice suggests a change at the cellular level. To address this question, we determined GR density in both groups. Western blot results and densitometry analysis presented in Fig. 1, C and D, show a dramatic increase in GR protein expression at day 1 postinjury in burned mice (P < 0.05). Modification in GR protein levels reached control values at days 2 and 5 following burn injury, demonstrating a transient and reversible modification.

Burn injury induced a selective change of 11β-HSD1 in thymus. Thymocytes from burn-injured mice have increased GR levels that correlated with greater sensitivity to CORT-induced apoptosis at day 1 postburn injury. Other cellular factors can influence the sensitivity to CORT. Particularly, it was previously demonstrated that TEC expressed 11β-HSD1 and that they were the main source of intrathymic GC (22). To demonstrate a possible change in 11β-HSD1 expression, we performed a Western blot analysis of total protein extracted from TEC isolated from sham- and burn-injured mice at day 1 postburn injury. Figure 2A shows that, on day 1, postburn injury 11β-HSD1 expression was not different between sham- and burn-treated animals. Although 11β-HSD1 levels were unchanged in TEC from burn-injured mice, we next wanted to know if thermal injury modulated 11β-HSD1 expression directly in thymocytes. First, we had to make sure that thymocytes were correctly isolated from TEC. For this purpose, we

A

B

C

D

Fig. 1. Thymocytes from day 1 burn-injured mice are more sensitive to corticosterone (CORT)-induced apoptosis and express higher levels of glucocorticoid receptor (GR). Thymocytes were isolated from mice at day 1 postburn injury and cultured in the presence of increasing concentrations (0.01–1 μM) of CORT. To specifically inhibit GR activity, CORT-exposed cells were previously treated with 100 nM RU-486. After cell culture, thymocytes were permeabilized, stained with propidium iodide (PI), and analyzed by fluorescence-activated cell sorter analysis (FACS). DNA sub-G₀ was an indicator of cell apoptosis. Results are shown as a percentage of cells that are positive for subdiploid DNA (±SE) (*P < 0.05) (A). FACS histograms are shown to demonstrate the difference between sham- and burn-treated cells (B). Independent experiments were performed 8 times. Western blot analysis of GR protein expression in thymocytes isolated from sham- and burn-injured mice on days 1, 2, and 5 postburn injury are shown. Bands shown correlate with molecular mass of ~95 kDa; one representative experiment out or three is shown (C). Results were normalized to actin and shown graphically (D) with each band; the vertical line represents the burn vs. sham ratio (±SE) (*P < 0.05).
It was shown that thymocytes were able to convert 11β-HSD1, resulting in amplifying the effect of both GR and 11β-HSD1 in thymocytes from burned mice had an increase of apoptosis. Figure 2 shows that thymocyte exposition to DHC (1 μM) led to significantly higher apoptosis (p < 0.05) compared with cells that were not exposed. Apoptosis levels of 1 μM DHC-exposed thymocytes were comparable to those of cells that were directly exposed to moderate amounts of active GCs, suggesting that DHC is partially converted to CORT and reached a concentration between 0.01 and 0.1 μM. To confirm CORT reactivation from DHC, thymocytes were incubated with DHC and the 11β-HSD1 inhibitor GA. Results show that GA halted apoptosis, suggesting an inhibition of reactivation of bioactive GC. Overall, 11β-HSD1 expressed in thymocytes acts as an oxoreductase on DHC.

11β-HSD1 expression in a thymoma cell line increases sensitivity to GC. It has been previously demonstrated that T cell hybridoma lines were sensitive to GC and had the capacity to determine in each preparation of cells the expression of VCAM-1, which is restricted to TEC in thymus (19). VCAM-1 expression was found in total thymus and TEC preparations but not in the thymocyte preparation (Fig. 2B), thus demonstrating that thymocytes were adequately isolated and free of any TEC contamination. Figure 2B shows also that total thymus, TEC, and isolated thymocytes expressed 11β-HSD1 mRNA. We next determined 11β-HSD1 mRNA expression in thymocytes from sham and burned mice at days 1, 2, and 5 postinjury. RT-PCR analysis of 11β-HSD1 mRNA levels showed a significant increase in thymocytes from burned mice at day 1 following injury (P < 0.01; Fig. 2, C and D). This increase was transient, since a normalization of 11β-HSD1 mRNA between two groups was observed at days 2 and 5 postburn injury (Fig. 2, C and D). These results demonstrate that increased 11β-HSD1 in thymocytes can represent another mechanism to amplify CORT action following a major injury and contributing to thymocyte depletion.

DHC conversion by 11β-HSD1 is responsible for thymocyte apoptosis. Thymocytes from burned mice had an increase of both GR and 11β-HSD1, resulting in amplifying the effect of CORT. It was shown that thymocytes were able to convert cortisone to cortisol through 11β-HSD1 activity (42). To determine the functionality of 11β-HSD1, thymocytes were isolated from healthy mice that had normal levels of GR and incubated in presence of DHC to evaluate CORT regeneration, leading to apoptosis. Figure 3 shows that thymocyte exposition to DHC (1 μM) led to significantly higher apoptosis (p < 0.05) compared with cells that were not exposed. Apoptosis levels of 1 μM DHC-exposed thymocytes were comparable to those of cells that were directly exposed to moderate amounts of active

![Fig. 2. Western blot analysis of 11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) protein expression in thymic epithelial cells (TEC) isolated from sham- and burn-injured mice is unchanged at day 1 postburn injury (A). Protein (5 μg) extracted from mouse liver samples was used as a positive control for 11β-HSD1 protein expression. Bands correlate with molecular mass of ~35 kDa. One representative experiment out of three is shown. 11β-HSD1 expression in thymocytes is increased at day 1 postburn injury. Total RNA was prepared from whole thymus TEC and isolated thymocytes from 3 mice and subjected to RT-PCR using primers for 11β-HSD1. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was amplified to visualize the amount of RNA loaded for each sample. Vascular cell adhesion molecule (VCAM)-1 mRNA expression, which is not present in thymocytes, was used as a control to verify any contamination of thymocytes from thymic structural components. Controls are performed with water instead of RNA or DNA, respectively, in RT-PCR and PCR reactions. 11β-HSD1 PCR product is represented by a band at 350 bp. 11β-HSD1 mRNA is expressed in whole thymus, TEC cells, and thymocytes (B). Increased mRNA levels are observed in isolated thymocytes from burn-injured mice at day 1 but not at days 2 and 5 postburn injury (C). Results were normalized to GAPDH and shown graphically (D) with each band; the vertical line represents the burn vs. sham ratio (±SE) (**p < 0.05).](http://ajpendo.physiology.org/doi/10.1152/ajpendo.00205.2008)
to generate cortisol from cortisone (42). We next wished to prove that DHC conversion to CORT in a thymic lymphoma cell line increased GC-induced apoptosis. First, we screened a panel of thymoma cell lines for 11β-HSD1 expression (data not shown). From this screening, we selected a representative cell line. The 267S3 thymic lymphoma cell line demonstrated the expression of 11β-HSD1 by RT-PCR (Fig. 4A). We then exposed these cells to 1 μM DHC and evaluated apoptosis 24 h later (Fig. 4B). Cell-cycle analysis showed a significant increase (p < 0.05) of thymoma that were apoptotic, and apoptosis induction was reversed when cells were preexposed to either GA or RU-486, thus demonstrating that DHC conversion to CORT was responsible for increased apoptosis. We also wished to determine if DHC conversion to CORT can be an additive phenomenon that could increase CORT-induced apoptosis in cells that were coexposed to a nonlethal concentration of CORT. 267S3 cells were exposed for 24 h to increasing amounts of CORT (0.01–1 μM) or 1 μM DHC. As expected, treatment of cells with 1 μM CORT exposure significantly increased cell apoptosis as did 1 μM DHC exposure (Fig. 4C). Interestingly, coexposure of cells to 0.01 μM CORT and 1 μM DHC led to increased apoptosis that was significantly higher than in cells treated solely with DHC. Increased apoptosis was not detected when cells were pretreated with the 11β-HSD1 inhibitor GA, demonstrating that intracellular generation of bioactive GC is responsible for increased sensitivity of cells to GC-induced apoptosis. Pretreatment of cells with GA did not prevent CORT-induced apoptosis, thus showing that this inhibitor was specific for 11β-HSD1 inhibition (Fig. 4D).

Proinflammatory cytokines increase thymocyte apoptosis through DHC conversion by 11β-HSD1. Several studies have demonstrated that proinflammatory cytokines, IL-1β, TNF-α, or IL-6, can increase 11β-HSD1 expression and activity (5, 10, 41). Major trauma and burn injury result in systemic inflammatory response syndrome, which involve the same cytokines. Because thymocytes from burn-injured mice upregulate both GR and 11β-HSD1 and are extremely sensitive to apoptosis induced by either TNF or GC treatment, we used thymocytes from control mice to determine if proinflammatory cytokines could increase 11β-HSD1 activity. We coexposed thymocytes to 1 μM DHC and either 1 or 10 nM of each of the previously mentioned cytokines. Cell apoptosis was evaluated 24 h later. Figure 5 shows that all three proinflammatory cytokines significantly increased DHC-induced apoptosis at the highest concentration (10 nM). Cells that were treated with 1 μM DHC and 1 nM of the cytokines showed similar apoptosis levels to cells treated with 1 μM DHC alone. Cytokines alone had no influence on cell apoptosis. This last result demonstrated that, as in other cell types, proinflammatory cytokines positively regulate 11β-HSD1 in thymocytes, since it increased DHC-induced apoptosis.

**DISCUSSION**

In this work, we showed that CORT sensitivity was regulated at the cellular level in thymocytes following a major trauma. We found that GR levels were upregulated in thymocytes at day 1 postburn injury, which correlated with increased sensitivity to CORT-induced apoptosis. Several reports have reported increased GR expression in target tissues when systemic stress hormone production was elevated (33), and it was...
It was reported that the thymus was able to produce GC locally through the expression of steroidogenic enzymes and that a complete pathway for GC biosynthesis from cholesterol existed within TEC (40). Thymocyte apoptosis induced by the cortical and medullary TEC line was related to a soluble factor (30). Zilberman et al. (43) identified this soluble factor as a steroid produced by TEC because double-positive CD4⁺CD8⁺ thymocyte apoptosis was inhibited when cells were exposed to GR inhibitor RU-486. Moreover, they demonstrated that low levels of GC produced by TEC seemed sufficient to alter both GR expression and sensitivity to GC in thymocytes (44). However, because thymic content of CORT is very low, on the order of 1:100,000 of the content of the adrenal, some data suggest that de novo GC production within the thymus is insufficient to exert GC signaling in thymocytes. Pruett and Padgett (26) have shown in adrenalectomized mice that locally produced GC within the thymus was insufficient to maintain normal levels of thymocyte apoptosis. In our model, we did not find any significant change in 11β-HSD1 expression in TEC on day 1 postburn injury. However, we did observe a significant increase directly in thymocytes from day 1 burn-injured mice. Increasing evidence has been compiled recently and demonstrates that 11β-HSD1 is expressed in mature T cells and thymocytes (42). Indeed, 11β-HSD1 expression in thymocytes begins during their maturation, more specifically when cells reach the cortico-medullary junction, and supports a role for GC in T cell development (22). In our model, we have found that thymocytes were able to convert DHC to CORT thus inducing their apoptosis. We were able to confirm DHC-induced apoptosis using the 267S3 thymoma cell line. We have shown an additive effect between CORT reactivation by 11β-HSD1 and CORT. When 267S3 cells were exposed concomitantly to DHC (1 μM) and a nondenleterious concentration of CORT (0.01 μM), apoptosis levels were higher than with cells treated with DHC (1 μM) alone, suggesting that DHC conversion to CORT is an additive event leading to increased intracellular CORT concentration and sensitivity to GC.

11β-HSD1 as been shown to be regulated by proinflammatory cytokines in various cell types (5, 12, 44). On the other hand, thymocytes are responsive to major proinflammatory cytokines, namely IL-1β, IL-6, and TNF-α (34). Because burn injury induces a major systemic proinflammatory storm as early as 6 h postburn (9, 12, 23), we have determined the influence of TNF-α, IL-1β, and IL-6 on DHC-induced apoptosis through 11β-HSD1 conversion. All proinflammatory cytokines tested increased DHC-induced apoptosis, suggesting an increase in expression levels and reductase activity of 11β-HSD1. Later effects have been observed in a rat embryonic cell line (10) and in adipocytes (14). Consequently, in an inflammatory condition associated with severe stress, the GC action can be amplified at the cellular level by an increase of 11β-HSD1 expression or activity under the influence of proinflammatory cytokines.

In this study, we reported high GR and 11β-HSD1 mRNA expression in thymocytes from day 1 burn-injured mice. We demonstrated that increased GR expression in the thymus led to thymic involution (25). GC are produced mainly by the adrenal glands in their bioactive form. Although 90–95% of the bioactive GC are bound to carrier proteins, mainly CBG, which controls their entry into cells, 11-keto metabolites such as cortisone and 11-DHC, which are biologically inactive, are able to diffuse freely in cells. 11β-HSD1 functions primarily as a reductase in cells, converting inactive 11-DHC into active CORT. We established for the first time the implication of local GC production in thymocytes in a situation where the systemic stress response is maximal.

![Graph A](image1.png)

**Fig. 5.** Proinflammatory cytokines increased DHC-induced apoptosis in thymocytes. Cells from control mice were cultured in the presence of 1 μM DHC and exposed to either 1 or 10 nM of interleukin (IL)-1β (A), tumor necrosis factor (TNF)-α (B), and IL-6 (C). Following cell culture, thymocytes were permeabilized, stained with PI, and analyzed by FACS. DNA subdiploidy was an indicator of cell apoptosis. Results are shown as a percentage of cells that are positive for subdiploid DNA (+SE) [⁎P < 0.05 vs. control (Crl); #P < 0.05 vs. DHC treated]. All experiments were repeated 8 times.
proved that 11β-HSD1 expression was sufficient to induce in vitro thymocyte apoptosis through the conversion of inactive 11-DHC to active CORT. We also showed that proinflammatory cytokines such as IL-1β, IL-6, and TNF-α, which are released in large amounts following thermal injury, increased GC-induced thymocyte apoptosis through CORT regeneration by 11β-HSD1. We therefore extend to thymocyte the report of Zhang et al. (42) that showed that 11β-HSD1 expression in lymphocytes provided a novel intracrine mechanism regulating GC activities, and we showed that in major trauma intracellular GC production might enhance thymocyte sensitivity to GC, thus leading to increased apoptosis and thymic involution. In the process of review of this manuscript, Qiao et al. (29) published a study showing that thymocytes express genes encoding for all enzymes required for de novo GC synthesis and their importance in the involution of thymus. Moreover, their study showed clearly that 11β-HSD1 expression was increased significantly with age in thymocytes. Our current study supports the importance of de novo GC synthesis in the involution of thymus after major injury and the influence of de novo synthesis on thymocyte homeostasis. The thymic involution process after burn injury needs to be further documented to allow a therapeutic intervention targeting de novo synthesis of GC, which contribute to decrease cellular immunity observed after major trauma.

GRANTS

The present study was supported by a grant from the Fondation des Pompiers du Québec pour les Grands-Brûlés. M. D’Elia and J. Patenaude were supported by a research award from the Fondation de la Recherche en Santé du Québec.

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