Expression of glucocorticoid resistance following social stress requires a second signal

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Abstract: Stimulation of splenocytes from socially stressed mice [social disruption (SDR)] with Gram-negative bacterial lipopolysaccharide (LPS) revealed a state of functional glucocorticoid (GC) resistance. LPS-stimulated splenocytes were less sensitive to the inhibitory effects of corticosterone. This study demonstrated that activation signals were required for the expression of splenic GC resistance. The results demonstrated that six cycles of SDR induced splenomegaly and increased the number of CD11b-positive monocytes. SDR also increased the viability of cultured, nonstimulated splenocytes, and addition of corticosterone reduced the viability of these cells in a dose-dependent manner. However, following stimulation with LPS, the sensitivity of SDR splenocytes to GC was reduced. Similar results were obtained using lipid A, a fraction of the LPS molecule that binds to Toll-like receptor (TLR)4. Furthermore, C3H/HeJ mice that do not possess a functional TLR4 molecule responded to SDR with an increased number of CD11b-positive monocytes in the spleen and increased viability of nonstimulated splenocytes. However, neither LPS nor lipid A stimulation resulted in the expression of GC resistance. Together, these findings suggest that the expression of GC resistance in response to SDR requires a second signal that can be provided by ligation of TLR4. J. Leukoc. Biol. 74: 507–513; 2003.

Key Words: social disruption (SDR) · lipopolysaccharide · monocytes/macrophages · CD11b · C3H/HeJ

INTRODUCTION

Chronic stress has long been known to alter innate- and adaptive-immune responses to a variety of pathogenic challenges [1]. Many of the immunosuppressive effects of stress were found to be mediated by the secretion of high levels of glucocorticoid (GC) hormones [1, 2]. Recently, we reported that social stress in mice altered the response of splenic mononuclear cells to GC. Specifically, following social disruption (SDR), lipopolysaccharide (LPS)-stimulated splenocytes were less sensitive to the inhibitory effects of corticosterone on cell proliferation and viability [3–5]. The development of GC resistance was accompanied by splenomegaly [3, 5] and a unique set of changes in the cellular phenotype in the spleen. Spleens from SDR mice contained higher numbers of mononuclear cells [3, 5]. Flow cytometry revealed that this augmentation in cell numbers was mainly a result of a large increase in the number of monocytes in the spleen. Additionally, spleens from socially stressed mice contained higher numbers of CD62L+ and CD11b+ monocytes [6]. LPS-stimulated splenocytes also secreted high levels of interleukin (IL)-6 in culture; however, this effect was sensitive to the inhibitory effect of GC [7]. The mechanisms involved in the development of SDR-induced GC resistance have not been fully elucidated. However, GC resistance was abolished by depletion of CD11b+ cells but not CD19+ cells from the cultures. This observation suggested a role for splenic monocytes/macrophages in GC resistance [5]. In addition, GC resistance was associated with reduced nuclear translocation of the GC receptor and with the inability of GC to suppress the activity of the inflammatory transcription factor, nuclear factor-kB, in monocytes [8].

The above-mentioned studies indicated that social stress altered the ability of macrophages to respond to activation signals such as LPS, resulting in increased cell survival in the presence of GC and augmented IL-6 production. The current study sought to further examine the function of splenocytes following SDR and the requirement for an activation signal such as LPS for the expression of GC resistance. In addition, as LPS was demonstrated to signal mainly through Toll-like receptor (TLR)4 [9, 10], we sought to further examine the involvement of this receptor in the expression of GC resistance in cells from SDR mice.

MATERIALS AND METHODS

Animals

Subjects were male C57BL/6, C3H/HeN (Charles River Inc., Wilmington, MA), or C3H/HeJ (Jackson Laboratories Inc., Bar Harbor, ME) mice, aged 6–8 weeks, and housed three to four per cage in an American Association for the Accreditation of Laboratory Animal Care-accredited facility. Mice were given free access to food and water and were maintained on a 12-h light/dark cycle (lights on at 6 a.m.). For the SDR stress, aggressive mice were also used. Aggressors were singly housed C57BL/6 male mice selected on the basis of...
pre-experimental screening for aggressive behavior. Screening entailed placing the aggressor into the home cage of another mouse. Only mice that initiated attacks within 5 min and showed consistent aggressive attacks toward their opponent were used in these experiments, Ohio State University Institutional Laboratory Animal Care And Use Committee approved animal care procedures.

Social Disruption Stress (SDR)

SDR was described previously [3]. Briefly, cages of male mice were randomly assigned into control or SDR groups. Control mice were housed in a different room from the SDR mice and remained undisturbed in their home cage. An aggressive mouse was introduced into the home cage of SDR mice for 2 h beginning at 4:30 p.m. Animals underwent six SDR cycles over a week: three nightly cycles, one night off, and three more cycles. Observations were made to ensure that the aggressors defeated the experimental subjects. Different aggressors were used on consecutive nights.

Splenocyte isolation and culture conditions

On the morning after the last SDR cycle, spleens were harvested, weighed, and placed in ice-cold Hanks’ balanced salt solution (HBSS). Spleens were pul- verized using a Seward Stomacher laboratory system to obtain single-cell suspensions. Red blood cells were eliminated by adding 1 ml lysis buffer (0.16 M NH₄Cl, 10 mM KHCO₃, 0.13 mM EDTA) for 2 min followed by one wash with HBSS/10% heat-inactivated fetal bovine serum (FBS). Each pellet was resuspended in HBSS, filtered through a sterile 70-μm nylon cell strainer to remove debris, and washed a final time in HBSS. Viable mononuclear cells were counted using an automatic cell counter (Z2, Beckman-Coulter, Miami, FL). An aliquot was set aside for flow cytometry analysis. Samples were resuspended (2.5x10⁴ cells/ml) in supplemented RPMI medium (10% heat-inactivated FBS, 0.075% sodium bicarbonate, 10 mM Hepes buffer, 100 U/ml penicillin G, 100 μg/ml streptomycin sulfamate, 1.5 mM L-glutamine, 0.00035% 2-mercaptoethanol). To stimulate the cells, LPS (Sigma L2630, Sigma Chemical Co., St. Louis, MO) or lipid A (diphosphoryl, Sigma L5399) was added at 2-mercaptoethanol). To stimulate the cells, LPS (Sigma L2630, Sigma Chemical Co., St. Louis, MO) or lipid A (diphosphoryl, Sigma L5399) was added at a concentration of 1 μg/ml. To control for mitogen stimulation, an additional aliquot of cell suspension was cultured in supplemented RPMI medium alone.

To test the sensitivity of cells to inhibition by glucocorticoids, aliquots from each cell suspension were treated with corticosterone (Sigma, C2505, from 0 to 5 μM), diluted in a buffer of 0.2% ethanol in supplemented RPMI. Cell suspensions were added in triplicate to flat-bottom, 96-well plates at a volume of 100 μl/well, and plates were incubated at 37°C and 5% CO₂. After 48 h, the cell viability assay was performed. Duplicate plates, containing 200 μl/well were cultured for 18 h. Supernatants from these plates were harvested and kept frozen in −70°C until assayed for IL-6 and tumor necrosis factor α (TNF-α).

Cell viability assay

The CellTiter 96 aqueous nonradioactive proliferation assay kit was purchased from Promega (Madison, WI). The tetrazolium substrate solution was prepared according to the instructions, and 20 μl was added to each well of the 96-well plates. Living cells convert this substrate to formazan, producing a brown precipitate. The plates were incubated at 37°C and 5% CO₂ for 3 h, and the resulting color changes were quantified by obtaining optical density (O.D.) readings at 490 nm on an enzyme-linked immunosorbent assay (ELISA) plate reader. To account for differences in background activity of cells, the mean O.D. of the three RPMI/FBS wells for a given treatment was subtracted from each of the corresponding stimulated values.

Flow cytometry

Total splenocyte numbers were obtained using an automatic cell counter (Z2, Beckman-Coulter). For flow cytometry, 2.5 x 10⁵ cells were incubated for 45 min at 4°C with antigen-presenting cell-conjugated anti-mouse CD11c (clone M1/70), fluorescein isothiocyanate-conjugated anti-mouse CD69 (clone H1- 20F3), phycoerythrin (PE)-conjugated anti-mouse CD62L (clone MEL-14), and PE-conjugated anti-mouse CCR5 (clone 2F3), phycoerythrin (PE)-conjugated anti-mouse CD62L (clone MEL-14), and PE-conjugated anti-mouse CCR5 (clone 2F3), respectively. Matched isotype controls were used to set negative-staining criteria for the quantitative analysis of CD62L, CD69, and CCR5 (CD195) expression on splenocytes.

Cytokine ELISA

IL-6

IL-6 concentrations in samples were measured by sandwich ELISA following a standard protocol. From BD PharMingen, Costar (Cambridge, MA), enzyme immunoassay (EIA)/radioimmunoassay (RIA) plates were coated with 50 μl/well 2 μg/ml anti-IL-6 (Clone MP5-20F3) overnight at 4°C. Plates were washed two times with PBS–TWEEN 20 and blocked with 200 μl/well PBS/10% FBS for 2 h at room temperature. Following three washes, 50 μl/well standards and samples were added and incubated overnight at 4°C. Plates were washed four times before adding 100 μl/well 1 μg/ml biotinylated anti-IL-6 (Clone MP5-32C11). After 1 h incubation at room temperature, plates were washed six times. Following addition of 100 μl/ml of a 1:1000 dilution of avidin-peroxidase (Vector, Burlingame, CA), plates were incubated at room temperature for 30 min. After eight washes, 100 μl/well 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) substrate (Sigma A-1836) was added, and the enzymatic reaction was allowed to develop at room temperature. O.D. was measured at 405 nm on an ELISA plate reader.

TNF-α

An OptEIASet mouse TNF-α (MonoPoly) ELISA from BD PharMingen was used to measure TNF-α concentrations following the manufacturer’s instructions. Briefly, Costar EIA/RIA plates were coated with 100 μl/well capture antibody (1:250 dilution) and incubated overnight at 4°C. Plates were washed three times and blocked with 200 μl/well PBS/10% FBS for 1 h at room temperature. Following three washes, 50 μl/well standards and samples were added and incubated for 2 h at room temperature. Plates were washed five times before adding 100 μl/well detection antibody (1:250 dilution) and incubating at room temperature for 1 h. After five washes, avidin-horseradish peroxidase (1:250 dilution) was added to plates at 100 μl/well, incubated at room temperature for 30 min, and washed again seven times. 3,3′,5,5′-Tetramethylbenzidine substrate reagent (BD PharMingen) was added 100 μl/well. After 15 min incubation in the dark, a 2N H₂SO₄ solution (50 μl/well) was added to stop the reaction. O.D. was measured at 450 nm on an ELISA plate reader.

IL-6 and TNF-α concentrations were quantified by using standard curves generated using serial dilutions of recombinant IL-6 and TNF-α (BD PharMingen), respectively. For statistical purposes, a sample falling below the detection limit of the ELISA was assigned the value corresponding to the sensitivity of that assay. The sensitivity range for IL-6 was 69–16,666 pg/ml and for TNF-α, 65–1,000 pg/ml.

Experimental design

Three strains of mice were chosen for this study based on their sensitivity to LPS stimulation: C57BL/6, C3H/HeN, and C3H/HeJ. The C3H/HeN and C3H/HeJ mouse strains diverged from the same progenitor strain, C3H/He, in 1947 [12]. C3H/HeJ mice possess a specific mutation in the TLR4 gene that results in a nonfunctional receptor and, therefore, are endotoxin-resistant [12]. The C3H/HeN strain possesses normal TLR4 on immune cells and exhibits vigorous responses to LPS [12]. Thus, this strain served as control for the effects of SDR on endotoxin-resistant mice. C57BL/6 mice also possess normal TLR4 molecules and were previously used to study the effects of SDR on splenocyte function [3–7]. Thus, C57BL/6 mice served as an additional control for this study. Cages of three to four mice of each strain were randomly assigned to home-cage control or SDR treatments (n=5 to seven/group). Control mice were left undisturbed in their cages until sacrificed. The rest of the mice underwent six cycles of SDR. All mice were sacrificed on the morning after the last stress cycle, and spleens were harvested, weighed, and processed for flow cytometry, cell viability assay, and cytokine secretion. Cells from mice of all strains were stimulated with LPS and with a specific mitogen for TLR4 (lipid A).
Statistical analysis

Spleen weight and cell numbers were analyzed using a two-way ANOVA, and stress (home-cage, SDR) and strain (C57BL/6, C3H/HeN, C3H/HeJ) were between subject factors. Cell viability and supernatant cytokine levels were analyzed using a three-way ANOVA, and stress (home-cage, SDR), strain (C57BL/6, C3H/HeN, C3H/HeJ), and corticosterone concentration (0–5 μM) were between subject factors. Fisher’s PLSD post-hoc test was performed to assess differences between experimental groups where appropriate. The level of significance was set at $P < 0.05$.

RESULTS

SDR induced splenomegaly in all three mouse strains ($F(1,30)=57.075; P<0.0001$; Fig. 1). Additionally, there was significant main effects for strain ($F(2,30)=3.704; P<0.05$), indicating that spleens from C3H/HeN– and C3H/HeJ–SDR mice were heavier than those of C57BL/6–SDR mice.

Single-cell suspensions were obtained from each spleen, and the number of leukocytes was determined. ANOVA revealed significant main effects for stress ($F(1,30)=49.766; P<0.0001$; Table 1) on leukocyte number, indicating that the number of splenocytes was significantly elevated by SDR in all three strains. Flow cytometry revealed a significant increase in the number of monocytes in the spleen, as well as in the number of monocytes expressing CD62L, CD69, and CCR5 (Table 1). Specifically, two-way ANOVA revealed significant main effects for stress ($F(1,30)=76.0, 77.19, 55.83, 35.0$ for total monocytes, CD62L$^+$, CD69$^+$, and CCR5$^+$ monocytes, respectively; $P<0.0001$), strain ($F(2,30)=7.81, 6.67, 7.95, 6.08$ for total monocytes, CD62L$^+$, CD69$^+$, and CCR5$^+$ monocytes, respectively; $P<0.01$), and an interaction between the effects of stress and strain ($F(2,30)=6.5, 6.17, 6.73, 5.1$ for total monocytes, CD62L$^+$, CD69$^+$, and CCR5$^+$ monocytes, respectively; $P<0.05$). Post-hoc analysis revealed that SDR elevated the total number of monocytes and CD62L$^+$ monocytes in all strains, and the number of CD69$^+$ and CCR5$^+$ monocytes in C3H/HeN and C3H/HeJ mice. In addition, in all categories, the number of cells in C57BL/6–SDR spleens was lower than in C3H/HeN– and C3H/HeJ–SDR groups.

Viability of nonstimulated cells was elevated by SDR, and corticosterone suppressed this effect in all strains (Fig. 2). There was significant main effects for strain, stress, and corticosterone ($F(2,144)=22.465; F(1,144)=89.419; F(4,144)=12.234$, respectively; $P<0.0001$), as well as an interaction between the effects of stress and strain ($F(2,144)=3.233; P<0.05$). Post-hoc analysis revealed that SDR increased viability of nonstimulated cells from all strains. Viability of cells from SDR mice was suppressed by the three highest concentrations of corticosterone in C3H/HeN mice.

| TABLE 1. Effect of SDR on Spleen Weight and Cell Composition in C57BL/6, C3H/HeN, and C3H/HeJ Mice |
|-----------------------------------------------|---------------|---------------|---------------|
|                  | C57BL/6       | C3H/HeN       | C3H/HeJ       |
|                  | HC            | SDR           | HC            | SDR           | HC            | SDR           |
| Spleen weight (mg) | 79.8 ± 4.5    | 158.8 ± 23.9$^*$ | 83.8 ± 1.6    | 228.9 ± 22.6$^*$ | 105.6 ± 6.2   | 234.2 ± 28.7$^*$ |
| Leukocyte number$^1$ | 77.2 ± 4.6    | 155.7 ± 29.9$^*$ | 55.1 ± 3.9    | 190.7 ± 22.1$^*$ | 186.6 ± 3.8   | 252.5 ± 31.7$^*$ |
| Monocyte number$^1$ | 1.1 ± 0.1     | 4.9 ± 1.3$^*$  | 1.1 ± 0.1     | 12.8 ± 1.5$^*$  | 1.9 ± 0.1     | 13.3 ± 2.1$^*$  |
| CD62L$^+$ monocytes$^1$ | 0.5 ± 0.1     | 4.1 ± 1.2$^*$  | 0.7 ± 0.1     | 10.6 ± 1.3$^*$  | 1.2 ± 0.2     | 10.9 ± 1.8$^*$  |
| CD69$^+$ monocytes$^1$ | 0.2 ± 0.1     | 1.3 ± 0.4     | 0.2 ± 0.1     | 4.4 ± 0.7$^*$   | 0.4 ± 0.1     | 5.4 ± 1.0$^*$   |
| CCR5$^+$ monocytes$^1$ | 0.1 ± 0.01    | 1.3 ± 0.4     | 0.1 ± 0.03    | 4.5 ± 1.2$^*$   | 0.4 ± 0.1     | 6.8 ± 1.4$^*$   |

$^1$ Cell numbers are expressed in millions. $^*$ Significantly different from same-strain HC (control) group ($P < 0.05$).
and by the two highest concentrations in C57BL/6 and C3H/HeJ mice. Viability of LPS-stimulated cells was increased by SDR in C57BL/6 and C3H/HeN mice compared with same-strain controls. SDR also induced GC resistance in these cultures (Fig. 3A). Specifically, ANOVA revealed significant main effects for strain, stress, and corticosterone [F(2,144)=63.556; F(1,144)=116.102; F(4,144)=34.552, respectively; P<0.0001], as well as an interaction between the effects of strain and corticosterone [F(8,144)=2.311; P<0.05]. Post-hoc analysis revealed that the viability of LPS-stimulated cells was significantly lower in C3H/HeJ compared with C57BL/6 and C3H/HeN, and SDR increased viability of cells from C57BL/6 and C3H/HeN. Additionally, the three highest concentrations of corticosterone suppressed viability of cells from control C57BL/6 and C3H/HeN, whereas viability of cells from SDR mice of these strains was suppressed only by the two highest corticosterone concentrations. In cells from C3H/HeJ mice, viability of cells from control mice was low, and SDR increased viability only in cell cultures without corticosterone or with the lowest (0.005 μM) corticosterone concentration. Viability of cells from C3H/HeJ-SDR mice was suppressed by the three highest corticosterone concentrations.

Viability of lipid A-stimulated cells was increased by SDR in C57BL/6 and C3H/HeN mice compared with same-strain controls. SDR also induced GC resistance in these cultures (Fig. 3B). Specifically, ANOVA revealed significant main effects for strain, stress, and corticosterone [F(2,138)=53.945; F(1,138)=78.096; F(4,138)=17.023, respectively; P<0.0001], as well as an interaction between the effects of strain and stress and between the effects of strain and corticosterone [F(2,138)=10.902; F(8,138)=2.481, respectively; P<0.05]. Post-hoc analysis revealed that viability of lipid A-stimulated cells was significantly lower in C3H/HeJ compared with C57BL/6 and C3H/HeN, as in cells from C3H/HeJ mice, viability of cells from control and SDR mice was low to undetectable. SDR increased viability of cells from C57BL/6 and C3H/HeN. Additionally, in C57BL/6, the three highest concentrations of corticosterone suppressed viability of control cells, whereas viability of cells from SDR mice was suppressed only by the two highest corticosterone concentrations. In C3H/HeN, the two highest concentrations of corticosterone suppressed viability of cells from controls, and only the highest corticosterone concentration suppressed viability of cells from SDR mice.

As our previous observations indicated that increased IL-6 secretion from splenocytes accompanied SDR-induced GC resistance [7], levels of IL-6 in the supernatant were measured in the current study (Fig. 4). In nonstimulated cultures, IL-6 levels were undetectable (data not shown). In LPS-stimulated cultures, ANOVA revealed significant main effects for strain, stress, and corticosterone [F(2,144)=8.308; F(1,144)=21.475; F(4,144)=4.477, respectively; P<0.005], as well as an interaction between the effects of strain and stress on IL-6 levels [F(2,144)=6.499; P<0.005]. In lipid A-stimulated cultures, ANOVA revealed significant main effects for stress and corticosterone [F(1,144)=28.92; F(4,144)=6.081, respectively; P<0.0005], as well as an interaction between the effects of stress and corticosterone [F(4,144)=4.383; P<0.005]. Post-hoc analysis revealed that in LPS and lipid A-stimulated cultures, SDR elevated IL-6 secretion from cells taken from C57BL/6 and C3H/HeN mice but not from C3H/HeJ mice. In addition, the three highest corticosterone concentrations suppressed IL-6 levels in cultures from C57BL/6- and C3H/HeN-SDR mice.

To assess whether the effects of SDR on IL-6 secretion were restricted to this cytokine, levels of another proinflammatory cytokine, TNF-α, were assessed (Fig. 5). TNF-α levels were measured in supernatants from splenocytes incubated with control medium, LPS, or lipid A. TNF-α was not detected in nonstimulated cultures (data not shown). In LPS-stimulated cultures, ANOVA revealed significant main effects for strain, stress, and corticosterone on TNF-α levels [F(2,144)=16.906; F(1,144)=105.76; F(4,144)=13.967, respectively; P<0.0001], as well as an interaction between the effects of strain and stress [F(2,144)=15.967; P<0.0001]. In lipid A-stimulated cultures, ANOVA revealed significant main effects for strain, stress, and corticosterone [F(2,138)=20.777; F(1,138)=43.658; F(4,138)=8.148, respectively; P<0.0001], as well as an interaction between the effects of strain and stress and the effects of stress and corticosterone on TNF-α levels [F(2,138)=13.458; F(4,138)=2.79, respectively; P<0.05]. Post-hoc analysis revealed that in LPS and lipid A-stimulated cultures, SDR increased TNF-α se-
cretion from cells taken from C57BL/6 and C3H/HeN mice but not from C3H/HeJ mice. In addition, the two highest corticosterone concentrations suppressed TNF-α levels in LPS-stimulated cultures, and the three highest corticosterone concentrations suppressed TNF-α levels in lipid A-stimulated cultures from C57BL/6 and C3H/HeN. The increased cytokine secretion was sensitive to the inhibitory effect of corticosterone, suggesting that the development of GC resistance was specific to a LPS-induced increase in cell viability. These findings are supported by our previous reports, which demonstrated the development of GC resistance and increased IL-6 secretion in splenocyte cultures from C57BL/6 following SDR [3–7].

The current study further showed that the ex vivo effects of SDR in endotoxin-resistant mice (C3H/HeJ) were different than in endotoxin-responsive mice. Much like in C57BL/6 and C3H/HeN, SDR induced splenomegaly and increased the number of splenic monocytes. The current study further demonstrated that these effects were evident in endotoxin-resistant (C3H/HeJ) and -responsive (C57BL/6 and C3H/HeN) strains of mice. Furthermore, following 48 h of incubation without stimulation, cell survival was increased by SDR in all three-mouse strains. Adding corticosterone to these cultures reduced viability of splenocytes, indicating that the cells were capable of respond-
C3H/HeN mice, C3H/HeJ mice responded to SDR with splenomegaly, increased number of splenic monocyte, and elevated cell survival in nonstimulated cultures. However, following LPS stimulation, GC resistance was not expressed in these cells, and cytokine levels were significantly reduced.

Together, these findings indicate that the development of GC resistance following SDR was a two-step process. The first stage, the priming of the cells, occurred in vivo and was characterized by a significant increase in the number of monocytes/macrophages in the spleen and as a result, an enlargement of the spleen. The phenotype of the monocytes was also altered; flow cytometry indicated a higher number of monocytes expressing the adhesion molecules CD62L and CD11b, the activation marker CD69, and the chemokine receptor CCR5. These cells also survived longer in culture without further mitogen stimulation. At this stage, these cells were still capable of responding to glucocorticoids, as addition of corticosterone to the nonstimulated cultures reduced cell survival. The second step of the development of GC resistance occurred in vitro. Spleen cells that were primed in vivo by SDR responded to stimulation with LPS with secretion of high levels of IL-6 and TNF-α compared with controls. Additionally, these cells lost their sensitivity to the inhibitory effects of corticosterone on cell viability. Survival of cells from SDR mice remained high compared with control cells even in the presence of high levels of corticosterone. These in vitro effects were not evident in C3H/HeJ mice, which are incapable of responding to LPS because of the mutation in the TLR4. This finding suggests that a second signal, such as LPS, was required for the expression of GC resistance in SDR splenocytes.

The signaling pathway of LPS has been described in great detail. The main receptor for LPS on mouse monocyte was shown to be TLR4 [13]. To further demonstrate the role of TLR4 ligation in providing the second signal, splenocytes were stimulated with lipid A, a specific mitogen for TLR4 [14]. Lipid A stimulation of SDR cells from an endotoxin-responsive strain resulted in high levels of cytokines and reduced sensitivity to corticosterone. In C3H/HeJ mice, lipid A did not affect cell function significantly. These findings indicate that LPS-induced GC resistance can be signaled via TLR4.

Several studies suggested a role for TLR2 as a receptor for LPS on mouse monocyte, particularly at high concentrations [15, 16]. The ability of LPS to signal through TLR2 may explain findings of LPS stimulation of cells from C3H/HeJ mice. LPS was capable of inducing low levels of IL-6 and TNF-α from cells from C3H/HeJ mice. LPS also induced a small increase in the viability of these cells (mainly in cells from the SDR group). The effects of LPS on the function of C3H/HeJ cells were minimal compared with its effects on cells from normal endotoxin-responsive mice. Therefore, the data may suggest that these effects were mediated by activation of TLR2 molecules on these cells.

The Toll protein was first described as a mediator in Drosophila innate immune system [14, 17]. Toll controls important antimicrobial responses against fungi and bacteria in the fruit fly. TLRs are the mammalian homologues of the Drosophila Toll receptor and were found to play a role in recognition of bacteria. In evolutionary terms, this protein family is a highly conserved signaling pathway that is a part of the first line of defense against infection or injury in invertebrates and vertebrates [17]. According to our findings, social stress altered the function of immune cells following TLR4 ligation. This social stressor, therefore, may have affected the process of bacterial recognition and altered the host response to infection or injury. It is important to note that although in the present study, this process occurred in a somewhat artificial system in vitro, it may also occur in the living animal following bacterial infection. A high level of aggressive behavior typically characterizes social stress in mice. Bite wounds are likely to appear following social stress and may contribute to the development of GC resistance [3]. As these wounds may be infected with opportunistic bacteria within hours of wounding [18], immune cells trafficking to the wound site are likely to encounter bacteria that may provide the second signal for the expression of GC resistance in vivo. Moreover, in a previous study, we examined the effects of SDR on mortality following an experimental model of sepsis [19]. In this model, injecting mice with high doses of LPS induced endotoxic shock. One of the known protective mechanisms against sepsis is the secretion of glucocorticoid hormones [20]. It is interesting that SDR greatly increased proinflammatory cytokine production (TNF-α and IL-1α) and mortality following LPS administration [19]. The mechanism by which SDR altered the response to endotoxic shock was not determined in this study. However, it was suggested that LPS reduced glucocorticoid sensitivity of immune cells in vivo, perhaps leading to enhanced, proinflammatory cytokine production. Thus, the protective effect of the glucocorticoid hormones was reduced, resulting in increased mortality [19]. This finding suggests that LPS may play a similar role in vivo and in vitro in altering the response of immune cells to bacterial products, resulting in reduced sensitivity to glucocorticoids.

Social stress-induced glucocorticoid resistance may have important clinical implications. GC resistance was reported to accompany several inflammatory diseases and was demonstrated to play a role in determining the course and prognosis of a variety of diseases [21, 22]. It has been demonstrated that endogenous glucocorticoids were not always effective in suppressing systemic inflammation, although circulating levels of cortisol were adequate [23]. Other reports indicated that in some patients, exogenous glucocorticoids had reduced potency in treating inflammatory diseases such as asthma, rheumatoid arthritis, and leukemia [24, 25]. Reports have associated this state of GC resistance with abnormalities in monocytes [25]. Animal models of stress-induced GC resistance, such as that described here, may contribute to an understanding of the mechanisms underlying inflammation-related changes in the sensitivity to glucocorticoids.

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