Sex-specific p38 MAP kinase activation following trauma-hemorrhage: involvement of testosterone and estradiol

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Angele, Martin K., Stefan Nitsch, Markus W. Knöferl, Alfred Ayala, Friedrich W. Schildberg, Karl W. Jauch, and Irshad H. Chaudry. Sex-specific p38 MAP kinase activation following trauma-hemorrhage: involvement of testosterone and estradiol. Am J Physiol Endocrinol Metab 285: E189–E196, 2003; 10.1152/ajpendo.00035.2003.—Although immune functions are markedly depressed in males and not in proestrous females following trauma-hemorrhage (T-H), the mechanisms responsible for the divergent responses remain unknown. Because sex steroids modulate the activation of p38, our aim was to determine whether testosterone and estradiol are involved in the activation of p38 by phosphorylation (p38-P) might contribute to the sex-dimorphic immune response following T-H. The effects of testosterone and estradiol on the activation of p38 were also examined. Intact male mice (C3H/HeN), castrated males treated with vehicle, 5α-dihydrotestosterone (DHT), or 17β-estradiol, and proestrous females were subjected to trauma (i.e., midline laparotomy) and hemorrhagic shock (35 ± 5 mmHg for 90 min and resuscitation) or sham operation. At 2 h thereafter, splenic (SMø) and peritoneal macrophages (PMø) were harvested and cultured (with 10 μg/ml LPS), and Western blot analysis was carried out for quantification of p38 and p38-P. Sex, testosterone and estradiol plasma levels, and T-H did not alter the constitutive expression of p38 in SMø and PMø. In contrast, the activated form of p38 (p38-P) was markedly increased in SMø and PMø from female shams compared with male shams. Moreover, the phosphorylation of p38-P increased in males after T-H, whereas it decreased in females under those conditions. Castration before T-H prevented the increase in p38-P in males. Castrated animals treated with DHT displayed increased p38-P following T-H, whereas 17β-estradiol had no effect on p38-P in castrated mice. Thus 1) sex influences the activation of p38 MAP kinase, 2) DHT is responsible for the increased activation of p38 in male mice, and 3) this sex-specific activation of p38 might be responsible for the sexually dimorphic immune response following T-H.

Several experimental studies demonstrate sex differences in the susceptibility to and morbidity from sepsis and trauma (3, 31, 33). Female mice in the proestrus state tolerate sepsis better than male mice, as evidenced by a significantly lower mortality rate following the induction of polymicrobial sepsis (33). The proestrus state of the estrous cycle is characterized by increased circulating estradiol levels (31). Furthermore, female mice in the proestrus state demonstrate maintained/enhanced immune responses as opposed to depressed immune function in males following trauma-hemorrhage (31). These findings are supported by several clinical studies demonstrating a preponderance of morbidity and mortality from sepsis in males compared with females (3, 19, 22, 23). Nonetheless, there is some inconsistency in the results of the clinical studies (3). The inconsistency in clinical studies might be because females, as studied in population studies, are in a mixed status of the menstrual cycle, therefore leading to inconsistency. Thus variations in the status of the menstrual cycle might account for the discrepancy in the findings between different clinical studies and experimental studies utilizing proestrous female rodents.

Testosterone and estradiol have been shown to mediate this sex-dimorphic immune response following adverse circulatory conditions (1–7, 29). In this respect, depletion of testosterone by castration before hemorrhagic shock prevented the depression of immune responses typically observed in intact males under those conditions (5, 6). Similarly, administration of an androgen receptor antagonist, e.g., flutamide, following trauma-hemorrhage in intact males restored the depressed immune responses and increased the survival rate of hemorrhaged animals subjected to subsequent sepsis (7, 29). In addition, administration of 5α-dihydrotestosterone (DHT) in female mice depressed the splenic and peritoneal macrophage immune response following trauma-hemorrhage to levels comparable to those of males under such conditions (1, 2). In contrast to testosterone, female sex steroids exhibit immunoprotective properties following hemorrhagic shock (6, 15).

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MATERIALS AND METHODS

Animals

Inbred male and proestrous female C3H/HeN mice (Charles River Laboratories, Sulzfeld, Germany, and Charles River Laboratories, Wilmington, MA), 7 wk old (24–26 g body wt), were used in this study. Six to eight animals were used for each experimental group. All procedures were carried out in accordance with the guidelines set forth in the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health. The Institutional Animal Care and Use Committees of the Regierung von Oberbayern and the Ludwig-Maximilians University, Munich, Germany and of Rhode Island Hospital and Brown University, Providence, RI, approved this project.

Experimental Groups

Male and proestrous female mice were randomized into four experimental groups and subjected to either trauma-hemorrhage or sham operation. To initially synchronize the estrous cycle of the females, the animals caged were kept together during the study period. The state of the estrous cycle was determined by examination of a vaginal smear before the trauma-hemorrhage procedure. Only proestrous female mice were included in the study, because sex-specific cytokine release capacities were most pronounced in this state of the estrous cycle (31).

In a separate set of animals, male mice were castrated 2 wk before trauma-hemorrhage or sham operation and treated with vehicle or physiological concentrations of DHT or 17β-estradiol. For this purpose, 21-day release pellets containing 7.5 mg of DHT, 0.5 mg of 17β-estradiol, or vehicle (Innovative Research of America, Sarasota, FL) were implanted subcutaneously with a 10-gauge trochar (Innovative Research of America) after the castration procedure. In previous studies, those pellets have been shown to cause physiological DHT and 17β-estradiol plasma levels in castrated mice (5, 6).

Trauma-Hemorrhage Procedure

Mice in the trauma-hemorrhage groups were lightly anesthetized with methoxyflurane (Metofane; Pitman-Moore, Mundelein, IL) and restrained in a supine position, and a 2.5-cm midline laparotomy (soft-tissue trauma) was performed, which was then closed aseptically in two layers using 6-0 Ethilon sutures (Ethicon, Somerville, NJ). After this, both femoral arteries were aseptically cannulated with polyethylene 10 tubing (Clay-Adams, Parsippany, NJ) by use of a minimal-dissection technique. Lidocaine was applied to all incision sites to prevent any discomfort to the animals, and the animals were then allowed to awaken. Blood pressure (BP) was constantly monitored by attaching one of the catheters to a blood pressure analyzer (Digi-Med, Louisville, KY). Upon awakening, the animals were bled rapidly through the other catheter to a mean arterial BP of 35 ± 5 mmHg (BP prehemorrhage was 95 ± 5 mmHg), which was maintained for 90 min. At the end of that period, four times the amount of shed blood was infused in the form of lactated Ringer solution to provide adequate fluid resuscitation. The catheters were then removed, the vessels ligated, and the groin incisions closed. Sham-operated animals underwent the same surgical procedure, which included ligation of both femoral arteries, but neither hemorrhage nor fluid resuscitation was carried out. There was no mortality observed in this trauma-hemorrhage model within the study period.

Blood and Cell Harvesting Procedure

The animals were killed by methoxyflurane overdose at 2 h after the completion of the trauma-hemorrhage procedure and resuscitation to obtain the spleen, peritoneal macrophages, and whole blood. Splenic and peritoneal macrophages from male and proestrous female mice were harvested 2 h after trauma-hemorrhage and resuscitation or sham operation. This time point was chosen because a sex-specific, LPS-induced cytokine release was observed in macrophages harvested 2 h after trauma-hemorrhage (31). The mice were killed at the same time of the day to avoid fluctuations of plasma hormone levels due to circadian rhythm.

Plasma Collection and Storage

Whole blood was obtained by cardiac puncture and placed in microcentrifuge tubes (Microtainer, Becton Dickinson, Rutherford, NJ). The tubes were then centrifuged at 16,000 g for 15 min at 4°C. Plasma was separated, placed in pyrogen-free microcentrifuge tubes, immediately frozen, and stored (−80°C) until assayed for DHT and 17β-estradiol.

Radioimmunoassays for Plasma DHT and 17β-Estradiol

Plasma DHT concentration was determined using a commercially available coated-tube radioimmunoassay (RIA) kit (Diagnostic Systems Laboratories, Webster, TX) in which 100-μl plasma samples were assayed in duplicate. The sensitivity of this RIA was 4 pg/ml. Plasma 17β-estradiol concentration was determined using a commercially available RIA kit (ICN Biomedicals, Costa Mesa, CA) in which 50-μl plasma samples were assayed in duplicate. The reactivity of both RIAs was 100% for their respective steroids.

Sex steroid plasma levels are presented as means ± SE. The one-way ANOVA method, followed by the Student-Newman-Keuls test or Tukey’s test as a post hoc test for multiple comparisons, was used to determine the significance of the differences between experimental means. A P value < 0.05 was considered to be significant.

Preparation of Spleenic Macrophage Culture

The spleens were removed aseptically and placed in separate Petri dishes containing cold (4°C) phosphate-buffered saline solution (PBS). The spleens were dissociated by grinding. The splenocyte suspension was used to establish macro-
phage cultures as previously described (6). The macrophage monolayers were stimulated for 15 min at 37°C, 5% CO₂, and 90% humidity with 10 μg of LPS/ml Click’s medium. In this respect, previous studies indicate a maximal p38 activation by phosphorylation (p38-P) in macrophages 15 min after LPS stimulation in vitro (16). Serum-free culture medium was used to avoid disturbance of protein quantification for Western blotting. At the end of the incubation period, the culture supernatants were disposed of, macrophages were lysed using RIPA buffer, and the cell lysate was stored at −70°C until assayed. RIPA buffer contained 100 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% deoxycholic acid, 1% Triton, 0.1% SDS, 0.05 ml of 250 mM sodium vanadate, and 0.2 ml of 25× proteinase inhibitor (Boehringer Mannheim, Mannheim, Germany).

Preparation of Peritoneal Macrophage Culture

Resident peritoneal macrophages were obtained from mice by lavaging the peritoneal cavity, and monolayers were established as previously described (6). This protocol provided adherent cells that were >95% positive by nonspecific esterase staining and that exhibited typical macrophage morphology. The macrophage monolayers were stimulated for 15 min at 37°C, 5% CO₂, and 90% humidity with 10 μg of LPS/ml Click’s medium. At the end of the incubation period, the culture supernatants were disposed of, macrophages were lysed using RIPA buffer, and the cell lysate was stored at −70°C until assayed.

Determination of Protein Levels in Cell Lysate

Protein levels in the cell lysate were measured using a commercially available colorimetric protein assay (Bio-Rad Laboratories, Hercules, CA) as described by the manufacturer. The reaction of this assay kit is similar to that of the Lowry method (18).

Determination of p38 and p38-P in Cell Lysate by Western Blot Analysis

In the lysate, p38 MAP kinase and the activation of p38 MAP kinase were measured by Western blot analysis. In brief, samples were electrophoresed using precast bis-tris minigels (2.6% approximate bis-acrylamide) from Novex (San Diego, CA). Proteins were transferred from the gels to polyvinylidene difluoride (PVDF) membranes by electroblotting using an X Cell II Mini Cell Gelbox (Novex, San Diego, CA). The membranes were reacted with anti-dual antibody-specific nonphosphorylated p38 (Pharmingen) or for p38-P (New England Biolabs). For the detection of bound antibodies, a chemiluminescence kit (Amersham Life Science) was used, and light emission was detected by exposure to sensitive autoradiography film. Three gels were run with 2–3 animals/group, and a representative gel is shown. For quantification, densitometry of activated p38 was performed with Sigma Gel software.

Statistical Analysis

The plasma hormones and the densitometry results of the Western blots are presented as means ± SE. One-way ANOVA, followed by the Student-Newman-Keuls test or Tukey’s test as a post hoc test for multiple comparisons, was used to determine the significance of the differences between experimental means. A P value <0.05 was considered to be significant.

RESULTS

Plasma DHT and 17β-Estradiol Levels

In castrated animals receiving vehicle, plasma DHT levels were found to be markedly decreased compared with the physiological plasma DHT levels detected in intact male mice (Table 1). Plasma DHT levels were significantly increased in DHT-treated castrated animals compared with vehicle-treated castrated male mice or proestrous female mice (P < 0.05) but were comparable to physiological plasma testosterone levels observed in intact male mice. The plasma DHT levels were not significantly different between sham-operated and corresponding trauma-hemorrhage groups.

Implantation of estradiol release pellets resulted in significantly increased plasma estradiol levels (P < 0.05) that were comparable to plasma estradiol levels in proestrous female mice (Table 2). Similar to plasma testosterone levels, trauma-hemorrhage did not alter plasma estradiol levels compared with the corresponding sham-operated animals.

Effect of Sex on Activation of p38

p38 MAP kinase expression in splenic macrophages. Sex as well as trauma-hemorrhage did not alter the constitutive expression of nonphosphorylated p38 in splenic macrophages (Fig. 1). In contrast, the activated form of p38, p38-P, was markedly increased in splenic macrophages harvested from female sham animals compared with male shams. However, the phosphorylation of p38 significantly increased in males that were subjected to trauma-hemorrhage (P < 0.05). In contrast, hemorrhaged female cells showed slightly decreased p38 phosphorylation compared with sham-operated females.

p38 MAP kinase expression in peritoneal macrophages. Nonphosphorylated p38 was comparable in male and sham-operated female animals in peritoneal macrophages (Fig. 2). After trauma-hemorrhage, the levels of nonphosphorylated p38 were similar to the levels in sham animals. In contrast, p38-P was increased in female shams compared with male sham animals (P < 0.05). The levels of p38-P significantly

Table 1. Plasma DHT levels from males and females and castrated male animals treated with vehicle, DHT, or estradiol

<table>
<thead>
<tr>
<th></th>
<th>DHT, pg/ml</th>
<th>Sham</th>
<th>Trauma-Hemorrhage</th>
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<tbody>
<tr>
<td>Male</td>
<td>2182 ± 284</td>
<td>1535 ± 370</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>170.8 ± 22.4</td>
<td>165 ± 25.8</td>
<td></td>
</tr>
<tr>
<td>Castrated male/vehicle</td>
<td>107.2 ± 6.9*</td>
<td>99.1 ± 6.7*</td>
<td></td>
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<tr>
<td>Castrated male/DHT</td>
<td>2567 ± 145</td>
<td>2103 ± 279</td>
<td></td>
</tr>
<tr>
<td>Castrated male/17β-estradiol</td>
<td>105.2 ± 6.9*</td>
<td>101.1 ± 7.4*</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as means ± SE. Male mice (C3H/HeN) were castrated and treated with either vehicle, 5α-dihydrotestosterone (DHT), or 17β-estradiol 2 wk before the experiment. Untreated male and female mice were also used. At 2 h after trauma-hemorrhage or sham operation (Sham), plasma DHT levels were measured by radioimmunoassay. Data were analyzed by ANOVA. *P < 0.05 vs. male Sham.
decreased in females following trauma-hemorrhage (<0.05), whereas they significantly increased in males under those conditions (P < 0.05).

Effect of DHT and Estradiol on Activation of p38

*p38 MAP kinase expression in splenic macrophages.* Castration, administration of DHT or 17β-estradiol, as well as trauma-hemorrhage did not alter the constitutive expression of p38 in splenic macrophages (Fig. 3). In contrast, the phosphorylation of p38 in splenic macrophages significantly increased in intact males following trauma-hemorrhage (P < 0.05). Castration before trauma-hemorrhage prevented the enhanced activation of p38-P in splenic macrophages harvested of those mice. Castrated mice treated with physiological amounts of DHT, however, displayed a significant increase in p38-P following trauma-hemorrhage (P < 0.05), comparable to that of intact males. Treatment of castrated mice with 17β-estradiol did not change

### Table 2. Plasma 17β-estradiol levels from males and females and castrated male animals treated with vehicle, DHT, or estradiol

<table>
<thead>
<tr>
<th>17β-Estradiol, pg/ml</th>
<th>Sham</th>
<th>Trauma-Hemorrhage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>27.2 ± 4.2</td>
<td>24.6 ± 3.3</td>
</tr>
<tr>
<td>Female</td>
<td>191.1 ± 37.1*</td>
<td>202.7 ± 42.6*</td>
</tr>
<tr>
<td>Castrated male/vehicle</td>
<td>33.2 ± 4.2</td>
<td>25.2 ± 6.4</td>
</tr>
<tr>
<td>Castrated male/DHT</td>
<td>44.3 ± 3.3</td>
<td>32.4 ± 3.7</td>
</tr>
<tr>
<td>Castrated male/17β-estradiol</td>
<td>227.2 ± 48.9*</td>
<td>217.4 ± 44.4*</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE. Male mice (C3H/HeN) were castrated and treated with either vehicle, DHT or 17β-estradiol 2 wk before the experiment. Untreated male and female mice were also used. At 2 h after trauma-hemorrhage or Sham, plasma 17β-estradiol levels were measured by radioimmunoassay. Data were analyzed by ANOVA. *P < 0.05 vs. male Sham.

![Fig. 1. Splenic macrophages harvested 2 h after trauma-hemorrhage (T-H) or sham operation (Sham) from male or proestrous female C3H/HeN mice. Splenic macrophages were cultured for 15 min in the presence of 10 μg/ml LPS. A: phosphorylation of p38 (p38-P) and constitutive p38 were determined by Western blot analysis. B: densitometry of activated p38 was performed with Sigma Gel software. Values are presented as means ± SE. Data were analyzed by ANOVA. *P < 0.05 vs. male Sham.](#)

![Fig. 2. Peritoneal macrophages harvested 2 h after T-H or Sham from male or proestrous female C3H/HeN mice. Peritoneal macrophages were cultured for 15 min in the presence of 10 μg/ml LPS. A: p38-P and constitutive p38 were determined by Western blot analysis. B: densitometry of activated p38 was performed with Sigma Gel software. Values are presented as means ± SE. Data were analyzed by ANOVA. *P < 0.05 vs. male Sham; #P < 0.05 vs. female Sham.](#)

![Fig. 3. Splenic macrophages harvested 2 h after T-H or Sham from male C3H/HeN mice that were sham castrated or castrated and treated with vehicle (Cast/Vehicle) or 5α-dihydrotestosterone (DHT) (Cast/DHT) for 2 wk before the experiment. Splenic macrophages were cultured for 15 min in the presence of 10 μg/ml LPS. A: p38-P and constitutive p38 were determined by Western blot analysis. B: densitometry of activated p38 was performed with Sigma Gel software. Values are presented as means ± SE. Data were analyzed by ANOVA. *P < 0.05 vs. male Sham; #P < 0.05 vs. Cast/DHT Sham.](#)
p38-P compared with vehicle-treated castrated animals (Fig. 4).

p38 MAP kinase expression in peritoneal macrophages. Similar to splenic macrophages, the expression of constitutive p38 was comparable in all study groups. The phosphorylation of p38, however, was markedly increased in peritoneal macrophages harvested from male mice following trauma-hemorrhage (Fig. 5). Castration increased the activation of p38 in sham-operated animals compared with intact males. After trauma-hemorrhage, the phosphorylation of p38 slightly decreased in castrated mice. This decrease, however, was not significant. Treatment of castrated mice with DHT decreased the activation of p38 in sham animals compared with vehicle-treated castrated mice (Fig. 6). A significantly increased phosphorylation of p38 was evident in DHT-treated castrated male mice following trauma-hemorrhage, similar to the pattern observed in intact male mice. 17β-Estradiol did not change the phosphorylation of p38 in castrated mice compared with vehicle treatment following sham operation or trauma-hemorrhage.

DISCUSSION

Previous studies have reported sex-specific immune responses following trauma-hemorrhage (31). Proestrus female mice show a maintained/enhanced immune response following trauma-hemorrhage compared with a depressed response in male mice (31). The underlying mechanisms for this sex-specific cytokine release following trauma-hemorrhage by splenic and peritoneal macrophages following LPS stimulation in vitro remain unknown. The activation of p38 MAP kinase in immune cells has been implicated in the...
control of the inflammatory response following shock (10, 11, 14, 21, 24). Thus the first aim of the present study was to determine whether differences in the activation of p38, as indicated by its phosphorylation (p38-P) soon after trauma-hemorrhage and resuscitation, might contribute to the sex-dimorphic immune response under such conditions.

The results of the present study demonstrate an increased activation of p38 in splenic and peritoneal macrophages harvested from males following trauma-hemorrhage compared with sham mice. In contrast, female mice exhibit increased p38 activation in sham animals, which decreased following trauma-hemorrhage. To the best of our knowledge, this is the first study investigating the effects of sex and male and female sex steroids on p38 activation following trauma-hemorrhage. Similar to trauma-hemorrhage, hypoxia led to increased p38 activation in male cardiac fibroblasts, whereas in females this effect was not evident (34). Those findings indicate that sex should be taken into account when signal transduction pathways following trauma-hemorrhage are studied.

It could be argued that the increased p38 activation in males following trauma-hemorrhage is associated with a decreased capacity to release proinflammatory cytokines by macrophages in vitro (6, 31). In contrast, proinflammatory cytokine RNA expression in splenic and peritoneal macrophages was increased following trauma and blood loss after 2 h of LPS stimulation (35, 36). Thus an increased activation of p38 in splenic and peritoneal macrophages of males following trauma-hemorrhage might result in an increased inflammatory cytokine response that is associated with an exhaustion of the capacity of those cells to release proinflammatory cytokines in vitro. In view of this, one would speculate that the suppressed p38 activation in females following trauma-hemorrhage results in decreased proinflammatory cytokine RNA expression, thereby maintaining cytokine release capacities in those animals. The hypothesis that p38 activation and cytokine gene expression correlate in splenic and peritoneal macrophages following trauma-hemorrhage, however, remains to be proven.

Studies have indicated that testosterone and estradiol mediate the sex-specific cytokine response following trauma-hemorrhage (1–7, 15, 30). Testosterone has been shown to exhibit immunosuppressive properties following trauma-hemorrhage, whereas 17β-estradiol is immunoprotective (1–7, 15, 30). In this respect, depletion of testosterone by castration 2 wk before trauma-hemorrhage prevented the depression of splenic and peritoneal macrophage cytokine release capacities (6, 30). Castrated mice supplemented with physiological levels of testosterone typically encountered in plasma, however, showed depressed macrophage functions following trauma-hemorrhage comparable to those of intact male mice (6). Similarly to castration, treatment of intact male mice following trauma-hemorrhage and resuscitation with the testosterone receptor antagonist flutamide resulted in maintenance of cytokine release capacities by splenic and peritoneal macrophages (7, 29). In contrast to treatment with male sex steroids, treatment of male mice with 17β-estradiol following trauma-hemorrhage restored the depressed splenic and peritoneal macrophages (15).

The precise mode of action of testosterone and estradiol on macrophage function following trauma-hemorrhage, however, remains unknown. In an attempt to elucidate the complex relationship of testosterone and estradiol on macrophage function following trauma-hemorrhage, male mice were castrated and supplemented with physiological amounts of DHT and/or 17β-estradiol by implantation of constant-release pellets in the present study. Administration of the androgen (DHT) and 17β-estradiol was chosen in this study because both sex hormones have been found to modulate immune responses following trauma-hemorrhage (2, 3, 15). Moreover, DHT and 17β-estradiol are considered to be the primary androgenic and estrogenic hormones in males and females. The plasma DHT and estradiol levels in treated castrated animals were found to be similar to physiological levels seen in intact male and proestrous female mice, respectively (1, 2). Trauma-hemorrhage did not significantly alter plasma sex steroid levels in the study groups.

The results indicate that castration of male mice prevented the increase in p38 activation in splenic and peritoneal macrophages following trauma-hemorrhage. Thus it is our hypothesis that testosterone is responsible for activating p38 signal transduction in males following trauma-hemorrhage. The present study also supports this hypothesis by illustrating that, by provision of DHT to castrated animals, the increased activation of p38 in splenic and peritoneal macrophages following trauma-hemorrhage could be reestablished. Unlike DHT treatment, treatment of castrated male mice with 17β-estradiol did not affect p38 activation following trauma-hemorrhage. Similarly, treatment of castrated male mice with 17β-estradiol did not alter the cytokine release of splenic and peritoneal macrophages following trauma-hemorrhage or sham operation (6). Although castrated male mice treated with 17β-estradiol showed no activation of p38, it remains unclear whether estradiol treatment during the different phase of the estrous cycle also decreases p38 activation in female animals. This aspect is particularly relevant because our findings indicate that proestrous females exhibit decreased p38 activation following trauma-hemorrhage compared with sham-operated females. Studies using female mice with low estradiol levels, i.e., mice in the diestrous state or gonadectomized females, are therefore needed to further elucidate the effect of estradiol on p38 in females. It is also possible that other hormones, e.g., progesterone, will also affect p38 activation in proestrous females after trauma-hemorrhage.

It should be noted, however, that several in vitro experiments confirm a divergent effect of estradiol and testosterone on signal transduction pathways (12, 20, 28). In this respect, the studies of Nuedling et al. (20) indicate that estradiol had no effect on the activation of
p38 in myocytes, whereas the activation of extracellular signal-regulated kinase (ERK)1/2 was increased in estradiol-treated cells. In a human neuroblastoma cell line, however, 17β-estradiol activated p38 as well as ERK1/2 (28). Guo et al. (12) demonstrated no effect of testosterone on the activation of p38 in intracellular androgen receptor-free murine RAW 264.7 macrophages. Addition of testosterone to the culture medium of LPS-stimulated androgen receptor-free RAW cells, however, increased the activation of p38 (12). In this experimental set-up, testosterone did not affect the activation of ERK1/2 (12). Those results indicate that testosterone and estradiol exhibit different effects on the activation of signal transduction pathways. Our studies focused on the activation of the p38 MAP kinase pathway. Further studies are required to elucidate the effect of sex and steroid hormone plasma levels on other signal transduction pathways, such as ERK1/2, to better understand the complex network of activated signals following trauma-hemorrhage. This is particularly important because trauma and shock activate various redundant signal transduction pathways that lead to similar cellular responses (17). In this regard, recent studies demonstrate an interaction between p38 and ERK (13). Inhibition of p38 has been shown to upregulate ERK activity in macrophages (13).

Depletion or administration of DHT had no effect on the cytokine release in sham-operated animals (2, 3, 7, 29). In contrast, castrated male mice displayed an increased activation of p38 in peritoneal and splenic macrophages harvested from sham animals compared with intact males. The increased p38 activation in castrated males was comparable to the pattern observed in sham-operated female mice. Those results suggest that testosterone also regulates the activation of p38 in sham-operated animals, whereas the cytokine release is not affected by sex hormones in those mice. This further illustrates that modulation of the p38 activation does not necessarily result in a modulation of the cytokine release capacities of macrophages.

The mechanisms by which DHT mediates its effect on p38 activation in splenic and peritoneal macrophages following trauma-hemorrhage remain unclear at present. Studies demonstrating that administration of the androgen receptor blocker flutamide restores the depressed immune responses following trauma-hemorrhage (7, 29) suggest, however, that immunomodulatory effects of DHT are mediated via the androgen receptors. In this respect, androgen receptors have been identified on macrophage-like synovial cells, immature monocyctic cells, and T and B cells (26, 32). Whether DHT produces p38 activation via binding to the androgen receptors on splenic and peritoneal macrophages remains to be determined. Studies using the androgen receptor antagonist flutamide in vitro might be useful in this regard. Alternatively, alterations in the p38 activation might be due to a sex-dimorphic activation of enzymes of sex steroid synthesis. In this respect, recent studies indicate a pivotal role of the enzymes of steroid synthesis in modulating immune responses (25, 27). However, determining which enzymes are involved in the modulation of p38 by DHT and estradiol following trauma-hemorrhage was beyond the scope of the present study.

In summary, the present study demonstrates that sex influences the activation p38 MAP kinase following trauma-hemorrhage, which may be related to the sexually dimorphic immune response under such conditions. Testosterone appears to be responsible for this sexual dimorphism, whereas 17β-estradiol has no effect on p38 activation. However, further studies using specific p38 MAP kinase blockers are required to determine whether differences in the p38 activation between males and females following trauma-hemorrhage are responsible for the sex-specific immune responses under those conditions. In addition, the effect of sex hormones on other signal transduction pathways, i.e., ERK1/2, should be investigated. The results of this study, however, suggest that sex and plasma steroid hormone levels should be taken into account when signal transduction pathways are studied following trauma-hemorrhage and other disease models. Moreover, p38 MAP kinase pathways may potentially represent a novel therapeutic target for the maintenance of immune function following severe trauma and blood loss.

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