Castration prevents suppression of MHC class II (Ia) expression on macrophages after trauma-hemorrhage

S. Mayr, C. R. Walz, P. Angele, T. Hernandez-Richter, I. H. Chaudry, F. Loehe, K. W. Jauch, and M. K. Angele. Castration prevents suppression of MHC class II (Ia) expression on macrophages after trauma-hemorrhage. J Appl Physiol 101: 448–453, 2006. First published April 13, 2006; doi:10.1152/japplphysiol.00166.2006.—Severe hemorrhage induces a severe depression of cell-mediated immune response (1, 2, 37). This depression is primarily mediated by a suppression of antigen presentation (23). In particular, T-cell activation requires antigen presentation via MHC class II (Ia) (21, 35). Inflammatory cytokines in response to LPS in vitro has been shown after trauma-hemorrhage in males (1, 2, 28). Castration of male mice 2 wk before trauma-hemorrhage resulted in maintained LPS-induced cytokine responsiveness of splenic and peritoneal MΦ (6, 7). Similarly, testosterone depletion restored the depressed Th1 cytokine release, i.e., IL-2, interferon (IFN)-γ, by T cells after trauma-hemorrhage (6).

Because antigen presentation is crucial for T-cell activation, maintained antigen presentation in castrated mice might contribute to improved T-cell responses in those animals. The aim of our study therefore was to determine if depletion of androgens by castration before hemorrhage has any salutary effects on the expression of major histocompatibility complex II on peritoneal and splenic MΦ after trauma-hemorrhage.

MATERIALS AND METHODS

Animals. Inbred C3H/HeN male mice (Charles River, Sulzfeld, Germany), between 5 and 7 wk of age were used in this study. 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 Committee of the Regierung von Oberbayern and the Ludwig-Maximilians University, Munich, Germany, approved this project.

Experimental groups. Male mice were randomized into four groups (n = 6–8 per group): group I, noncastrated, sham operated (no trauma, no hemorrhage); group II, castrated, sham operated (no trauma, no hemorrhage); group III, noncastrated, trauma-hemorrhage; group IV, castrated, trauma-hemorrhage.

Castration procedure. Mice were castrated 2 wk before the experiment as previously described (7). In brief, mice were anesthetized with isoflurane (Forene, Abbott, Wiesbaden, Germany), restrained in a supine position, and the skin of the scrotum was disinfected with isopropyl alcohol (70%). A midline incision of about 3 cm was made, and the testes were exposed. The scrotal incision was closed using 5–0 Ethilon (Ethicon, Hamburg, Germany). Mice in groups I and III

HEMORRHAGIC SHOCK induces a severe depression of cell-mediated immune response that is associated with an increased susceptibility to sepsis (9, 10, 41). In this respect, the capacity of splenic and peritoneal macrophages (MΦ) to release proinflammatory cytokines in response to LPS in vitro has been found to be depressed after trauma-hemorrhage in male mice (6, 7). Furthermore, altered antigen presentation has been shown after trauma-hemorrhage in males (10). Presentation of foreign antigens by antigen-presenting cells, however, is essential for initiating and maintaining cell-mediated immune responses (21, 35). In particular, T-cell activation requires antigen presentation via myosin heavy chain (MHC) class II (13, 15). Similarly, in clinical studies, diminished capacities of peripheral blood mononuclear cells to present antigen as measured by MHC class II expression after major abdominal surgery resulted in higher infection (36) and mortality rates (27). Moreover, sex dimorphism in the immune and organ response, and the susceptibility to morbidity and mortality from trauma and hemorrhagic shock, have been demonstrated. Sex hormones have been shown to contribute to this sex-dimorphic immune response after adverse circulatory conditions (1, 2). Specifically, studies indicate that androgens are responsible for the immunodepression after trauma-hemorrhage in males (1, 2, 28). Castration of male mice 2 wk before trauma-hemorrhage resulted in maintained LPS-induced cytokine responsiveness of splenic and peritoneal MΦ (6, 7). Similarly, testosterone depletion restored the depressed Th1 cytokine release, i.e., IL-2, interferon (IFN)-γ, by T cells after trauma-hemorrhage (6).

Because antigen presentation is crucial for T-cell activation, maintained antigen presentation in castrated mice might contribute to improved T-cell responses in those animals. The aim of our study therefore was to determine if depletion of androgens by castration before hemorrhage has any salutary effects on the expression of major histocompatibility complex II on peritoneal and splenic MΦ after trauma-hemorrhage.

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**RESULTS**

**CD11b (Mac-1): expression on splenic and peritoneal MΦ.** In an attempt to verify the percentage of MΦ harvested with the immune cells, CD11b (Mac-1), a macrophage marker, was determined. The results indicate that the expression of CD11b on splenic (Fig. 1A) and peritoneal MΦ (Fig. 1B) was similar in castrated and sham-castrated mice. Moreover, the expression of CD11b was not affected by the trauma-hemorrhage procedure.

**MHC class II (Ia): expression on splenic MΦ.** Trauma-hemorrhage resulted in a significant suppression of MHC class II. A higher percentage of MΦ expressed MHC class II (Ia) antigen following trauma-hemorrhage and resuscitation in noncastrated mice with respect to sham-operated and castrated mice. The expression of MHC class II (Ia) was significantly lower in mice undergoing trauma-hemorrhage and resuscitation compared to sham-operated and castrated mice (Fig. 1C). These results were consistent with previous observations in trauma-hemorrhage models. The suppression of MHC class II (Ia) expression in trauma-hemorrhage and resuscitation was not observed in castrated mice, suggesting that castration prevents the suppression of MHC class II (Ia) following trauma-hemorrhage and resuscitation.

**Statistical analysis.** The ANOVA analysis was performed to determine the significance of the differences between experimental groups. A post-hoc test was used to compare the results within each experimental group. The results indicated that the expression of MHC class II (Ia) was significantly different between experimental groups, with the highest expression observed in non-castrated mice and the lowest expression in sham-operated mice.

**Fig. 1.** CD11b expression on splenic (A) and peritoneal macrophages (MΦ; B) harvested 4 h after trauma-hemorrhage (groups III and IV) or sham operation (not traumatized and not shocked; groups I and II) from male C57/12N mice that were either castrated (groups II and IV) or sham castrated (groups I and III) 2 wk before the experiment. CD11b expression was analyzed by flow cytometry. Values are presented as means ± SE; n = 6 or 7/group. Positive.
II (Ia) expression on splenic MΦ in sham-castrated animals (group IIII) compared with sham-castrated mice that were not subjected to trauma-hemorrhage (−19.9% group III vs. group I, P < 0.05; Fig. 2). Castration significantly increased the expression of MHC class II (Ia) expression on splenic MΦ after trauma-hemorrhage compared with sham-castrated trauma-hemorrhaged mice (30% group IV vs. group III, P < 0.05). Castration, however, did not affect MHC class II (Ia) expression on splenic MΦ harvested from sham-operated (groups I and II; not traumatized and not shocked) mice (Fig. 2). The representative histograms are shown in Fig. 3, A and B.

MHC class II (Ia): expression on peritoneal MΦ. Similarly, the expression of MHC class II (Ia) expression on peritoneal MΦ significantly decreased in sham-castrated mice compared with sham-castrated mice that were not subjected to trauma-hemorrhage (−38.7% group III vs. group I, P < 0.05; Fig. 4). Castration 2 wk before trauma-hemorrhage restored the depressed MHC class II (Ia) expression on peritoneal MΦ after trauma-hemorrhage compared with sham-castrated trauma-hemorrhaged mice (89.5% group IV vs. group III, P < 0.05). Interestingly, castration did not affect MHC class II (Ia) expression on peritoneal MΦ from sham-operated (groups I and II; not traumatized and not shocked) mice (Fig. 4). The representative histograms are shown in Fig. 5, A and B.

DISCUSSION

Several clinical and experimental studies have demonstrated depressed cell-mediated immune responses after trauma and blood loss (4, 18, 19, 42). In particular, a defective capability of MΦ to present foreign antigens after trauma and blood loss (10, 18, 36) results in compromised initiation of an adoptive immune response. In this respect, compromised antigen presentation has been associated with depressed T-cell function (21, 35). Moreover, the depression of antigen presentation results in an increased infection rate and poor outcome after major surgery (18, 24, 36). On the basis of those findings, the crucial role of MHC class II in initiating an adequate immune response has been extrapolated, although this has not been investigated in the current study.

Recently, an important role of sex in mediating immune responses after trauma-hemorrhage has been established (3, 16, 40). Male mice exhibit suppressed immune responses, whereas female mice in the proestrus state do not show such a depression (40). Thus male sex hormones have been shown to be responsible for producing this immunosuppression in males (6, 7). Interestingly, androgen receptor blockade by flutamide after trauma-hemorrhage resulted in a decreased susceptibility to subsequent polymicrobial sepsis (9). Although it could be speculated that depletion of testosterone will prevent depression of antigen presentation and thereby maintain bacterial defense mechanisms, this aspect has not yet been examined. To study this, male mice were castrated 2 wk before trauma-hemorrhage or sham operation. In several previous studies, castration of male mice 2 wk before the experiment has been shown to significantly lower plasma testosterone levels by ~95% (2, 6, 26, 39). This reduction in testosterone plasma levels after castration was comparable in all studies (2, 6, 26, 39). In view of this previous work, testoster-
Values are presented as mean ± SE, n = 6 or 7/group. Data were analyzed by ANOVA. *P < 0.05 vs. group I; #P < 0.05 vs. group III.

one levels in the plasma were not determined in the present study to avoid redundancy.

The results of the present study indicate depressed MHC class II (Ia) expression by splenic and peritoneal MΦ harvested from intact male mice after trauma-hemorrhage. This is in agreement with previous findings demonstrating decreased MHC class II (Ia) expression in MΦ harvested from various compartments after trauma-hemorrhage in untreated male mice (10). Sham-castrated, sham-trauma-hemorrhaged mice (group I), which do not represent completely untreated animals, served as controls in the present manuscript. Nonetheless, MHC class II (Ia) expression on sham-castrated sham-trauma-hemorrhaged animals (group I) were similar to values reported previously in completely untreated C3H/HeN mice (11, 17). Thus the sham-castration procedure 2 wk before the experiment did not affect MHC class II (Ia) expression. The results further suggest that the decrease in MHC class II expression after trauma-hemorrhage in peritoneal MΦ might be due to redistribution among two different cell populations, i.e., dendritic cells and MΦ. In contrast, in splenic MΦ a leftward shift in fluorescence intensity is evident. This discrepancy appears to be due to the different compartments from which the cells were harvested, i.e., peritoneum vs. spleen.

The present study extended that observation and also indicated that castration of animals before trauma-hemorrhage prevented the depression of MHC class II (Ia) expression by splenic and peritoneal MΦ. Previous findings suggest that depletion of 5α-dihydrotestosterone in castrated males is responsible for the improved immune responses after trauma-hemorrhage in those animals (2). In this respect, treatment of castrated males with 5α-dihydrotestosterone resulted in suppressed splenic and peritoneal MΦ cytokine responses after trauma-hemorrhage comparable to noncastrated male mice (7, 8). Thus one would speculate that the testosterone derivate 5α-dihydrotestosterone also plays a pivotal role for suppressing MHC II (Ia) expression. This hypothesis, however, needs to be verified in additional studies using castrated male mice treated with physiological amounts of 5α-dihydrotestosterone.

Alternatively, the use of a specific testosterone receptor blocker, i.e., flutamide, in normal male mice would further clarify the role of testosterone in suppressing MHC class II expression after trauma and blood loss. Additional support for our results comes from the studies of Weinstein et al. (37), which showed that in vitro primed MΦ harvested from female mice are more efficient than male cells in initiating a secondary response in lymphocytes. Their study also showed that castration of male mice enhanced, whereas administration of 5α-dihydrotestosterone in female mice reduced, the efficiency of antigen presentation by primed MΦ (37).

The percentage of CD11b-positive cells, a marker of macrophage activation, was unaffected by trauma-hemorrhage or castration between the study groups. The results further indicate, however, a disparity in the percentage of CD11b-positive cells between splenic and peritoneal MΦ, demonstrating a compartment-dependent variation between the spleen and the peritoneum. These findings are in accordance with previous studies (34). Moreover, the activation status of harvested MΦ was similar in all study groups as indicated by a comparable CD11b expression (34). T cells might depress MΦ functions in the spleen, resulting in lower CD11b levels after trauma-hemorrhage. In addition, the number or viability of MΦ obtained from the peritoneum or the spleen has been shown to be similar in hemorrhaged and sham-operated mice in previous studies (10). These findings suggest that the decreased percentage of MHC II (Ia) expression in hemorrhaged mice is not due to variations in cell distribution or loss of viability. Because

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**Fig. 4.** MHC class II (Ia) expression on peritoneal MΦ harvested 4 h after trauma-hemorrhage (groups III and IV) or sham operation (not traumatized and not shocked; groups I and II) from male C3H/HeN mice that were either castrated (groups II and IV) or sham castrated (groups I and III) 2 wk before the experiment. MHC class II (Ia) expression was analyzed by flow cytometry. Values are presented as mean ± SE, n = 6 or 7/group. Data were analyzed by ANOVA. *P < 0.05 vs. group I; #P < 0.05 vs. group III.

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**Fig. 5.** Representative histograms of MHC class II (Ia) expression on peritoneal macrophages harvested 4 h after trauma-hemorrhage (groups III and IV; shaded line) or sham operation (not traumatized and not shocked; groups I and II; solid line). A: sham castrated (groups I and III). B: castrated (groups II and IV). Macrophages were analyzed by flow cytometry.
dendritic cells capable of expressing MHC class II also stick to culture plates, dendritic cells might be included in the analyzed cell suspension. This is a limitation of the present study, and therefore further studies are required to investigate the effect of trauma-hemorrhage and castration on separated antigen-presenting cells.

Interestingly, castration of male mice did not alter MHC II (Ia) expression in sham-castrated mice. However, depletion of testosterone by castration restored the depressed cytokine release capacity of splenic and peritoneal MΦ after trauma-hemorrhage, whereas cell-mediated immune responses in sham-castrated mice were not affected (7, 39). These findings suggest that physiological levels of testosterone are only harmful in an immunologically compromised host but not in normal animals. Differences in the kinetics of testosterone might explain the immunosuppressive effects of physiological testosterone plasma levels in those animals. In this regard, the sex steroid synthesis has been shown to be altered after hemorrhage (31, 32, 44). Those studies demonstrate increased intracellular levels of 5α-dihydrotestosterone and decreased catalysis of this steroid hormone after trauma-hemorrhage due to alteration in the activity of enzymes involved in the steroid synthesis (31, 44). In particular, 5α-reductase activity was increased, whereas 17β-hydroxysteroid dehydrogenase activity decreased, after trauma-hemorrhage in lymphocytes harvested from male mice. In accordance with our findings, other studies have also shown that androgens do not depress MΦ cytokine release from normal animals (3, 7, 29).

In the present study, MΦ antigen presentation per se was not determined. Nonetheless, the expression of MHC class II (Ia) has been shown to correlate with the capacity of MΦ to present antigens (10, 11). In this respect, Ayala et al. (10, 11) demonstrated that the diminished antigen presentation capacity of peritoneal and spleen MΦ after trauma-hemorrhage was due to the loss of MHC class II (Ia) expression (10, 11). In addition, T-helper cell activation requires presentation of foreign antigens via MHC class II (13, 15, 33). The decreased expression of MHC class II by antigen-presenting cells leads to severe immunodeficiency (25). In clinical studies, diminished MHC II expression was associated with increased infection and mortality rates (18, 24, 36). Thus one would speculate that diminished MHC class II is associated with defective antigen presentation. Further studies, however, are required to validate this notion.

MHC II (Ia) measurements were restricted to 4 h after trauma-hemorrhage. It should be noted that an early restoration of immune responses in our trauma-hemorrhage model was associated with prolonged protection against subsequent sepsis (4, 9, 22, 38). In this regard, studies indicate that restoration of immune responses 4 h after trauma-hemorrhage due to testosterone depletion is associated with increased survival after the induction of subsequent sepsis on the third postoperative day (9). Thus it appears likely that MHC II (Ia) expression is improved for a prolonged time after trauma-hemorrhage in castrated mice. Additional studies, however, are required to verify this notion.

The exact underlying mechanism for the lack of depression in MHC II (Ia) expression in castrated animals remains unknown. Multiple factors that include decreased metabolic activity, anti-inflammatory cytokines, prostaglandins, and nitric oxide appear to be responsible for producing the depression in MΦ antigen presenting capacity (5). A moderate inflammation in testosterone-depleted animals, i.e., release of IL-6 and TNF-α, as opposed to an excessive inflammatory response in noncastrated males after trauma-hemorrhage has been previously demonstrated (3, 7). This effect of castration might contribute to the maintained MHC II (Ia) expression in castrated mice (3, 7). In this respect, beneficial effects of low levels of circulating TNF-α on MΦ MHC II (Ia) expression have been shown (14). Alternatively, defective T-cell responses, i.e., depressed IL-2 and IFN-γ release, in noncastrated male mice (6) might be the cause for diminished MΦ functions. Administration of INF-γ in vitro and in vivo markedly improves the expression of MHC class II antigen after hemorrhage in mice and severely injured patients (10, 20). Although several parameters in castrated mice after trauma-hemorrhage have been determined, the precise mediator that directly affects MHC class II expression has not been identified. Moreover, it remains unknown whether castration exerts its effects on MHC class II expression through genomic or nongenomic mechanisms. In this respect, recent studies indicate that decreased MHC II RNA expression is responsible for suppressed MHC II expression after septic shock (30). Those studies therefore suggest that suppression of MHC class II after shock is due to alterations at the transcriptional level. In summary, the precise mechanism by which MHC class II expression is decreased after trauma-hemorrhage and how depletion of testosterone prevents its decrease was, however, beyond the scope of the present study.

Our results indicate that MHC II (Ia) expression on splenic and peritoneal MΦ was impaired after trauma-hemorrhage in intact male mice. Castration of male mice 2 wk before trauma-hemorrhage prevented the depression in MHC II (Ia) expression. Although the effect of an androgen receptor blocker on MHC II (Ia) expression has not been evaluated, previous findings suggest that short-term treatment with flutamide after trauma-hemorrhage might also beneficially influence MHC II (Ia) expression (9, 38). Thus attempts to improve MHC II (Ia) expression after trauma-hemorrhage by treatment with androgen receptor blockers might represent useful adjunct for maintaining innate and adaptive immunity and for decreasing the incidence of infections in surgical patients.

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REFERENCES


