Impact of sex and age on bone marrow immune responses in a murine model of trauma-hemorrhage

Christian P. Schneider, Martin G. Schwacha, and Irshad H. Chaudry

Center for Surgical Research, Department of Surgery, University of Alabama at Birmingham, Birmingham, Alabama

Submitted 1 August 2006; accepted in final form 3 October 2006

Schneider CP, Schwacha MG, Chaudry IH. Impact of sex and age on bone marrow immune responses in a murine model of trauma-hemorrhage. J Appl Physiol 102: 113–121, 2007. First published October 5, 2006; doi:10.1152/japplphysiol.00848.2006.—Although studies have demonstrated that trauma markedly alters the bone marrow immune responses, sex and age are crucial determinants under such conditions and have not been extensively examined. To study this, 21- to 27-day-old (premature), 6- to 8-wk-old (mature), and 20- to 24-mo-old (aged) male and female (proestrus) C3H/HeN mice were sham operated or subjected to trauma (i.e., midline laparotomy) and hemorrhagic shock (30 ± 5 mmHg for 90 min) followed by fluid resuscitation. Twenty-four hours after resuscitation, bone marrow cells were harvested. Trauma-hemorrhage induced an increased number of the early pluripotent stem cell-associated bone marrow cell subsets (Sca1CD34CD117lin) in young mice. The CD117+ proportion of these cell subsets increased in mature proestrus females, but not in males. Aged males displayed significant lower CD117lin cells compared with young male mice. Trauma-hemorrhage also increased development of granulocyte/macrophage progenitor cells (CD11bGr-1+). Proliferative responses to granulocyte macrophage colony-stimulating factor were maintained in mature and aged proestrus females, but decreased in young mice and mature males. Augmented differentiation into monocyte/macrophage lineage in mature and aged proestrus females was observed and associated with the maintained release of TNF-α and IL-6. Conversely, increased IL-10 and PGE2 production was observed in the male trauma-hemorrhage groups. Thus, sex- and age-specific effects in bone marrow differentiation and immune responses after trauma-hemorrhage occur, which are likely to contribute to the sex- and age-related differences in the systemic immune responses under such conditions.

Sepsis and subsequent multiple organ dysfunction syndrome (MODS) are the most common causes of death in the surgical intensive care units (5). Injury-induced anergy of the immune system has been postulated to be the major factor in the high susceptibility of trauma patient to serious infection and subsequent MODS (1, 7, 11, 23). Bone marrow failure is one facet of the MODS and is commonly seen in patients recovering from severe trauma and hemorrhagic shock (15). Earlier experimental studies have indicated that hemorrhagic shock, acute hypoxia, and endotoxin led to impaired bone marrow proliferative capacity, in particular the growth of granulocyte/macrophage progenitor cells (25, 26). In contrast, Santangelo et al. (41) reported recently that the bone marrow is driven to monocytopoiesis following thermal injury and infection in mice. Additionally, Moore et al. (35) demonstrated a decreased ability of peripheral mononuclear cells to support bone marrow growth in patients following severe torso trauma. Although traumatic injury appears to be associated with bone marrow alterations, the mechanisms by which this occurs remain to be fully determined.

Both clinical and experimental studies demonstrated that sex and age are crucial determinants in host responses following traumatic injury, shock, and infection (2, 19, 30, 43). Aging and the male sex are associated with a higher risk of mortality after a traumatic/infectious event (32, 34, 42, 51, 54). It is known that aging causes a decrease in the number of naive T cells and increased accumulation of memory T cells and a predominance of a T-cell helper type 2 phenotype (i.e., IL-4, IL-10) as opposed to a T-cell helper type 1 phenotype (i.e., IL-2, IFN-γ) in younger individuals (24, 34). With respect to hematopoietic function, current evidence suggests that aging-related bone marrow changes are rather subtle (36, 38). Nonetheless, such changes may become clinically evident under conditions of severe hematopoietic stress, such as repeated courses of chemo-radiotherapy. No information is available on how these alterations impact bone marrow response to trauma, shock, and infection. For immature individuals, it has been shown that those who require massive surgical interventions are particularly vulnerable to infections due to immaturity of the immune system (16). It is known that bone marrow cellularity declines with age until the age of 5 yr in humans and then remains stable (12). Nonetheless, hematopoietic recovery is more rapid in children with fewer problems and complications (52).

In addition to the effects of age on immune function, it has been demonstrated that young males exhibit depressed immune responses and increased susceptibility to sepsis following trauma-hemorrhage, whereas proestrus females, with elevated estrogen levels, have maintained immune responses under such conditions (15). Sex-related differences have also been observed by other investigators showing an estrogen-mediated increase in glutathione peroxidase erythrocyte activity in females, and for males an increased susceptibility to infection with murine leukemia virus and skin tumor susceptibility (4, 6, 29). Lea et al. have shown androgen-mediated depression of macrophage colony-stimulating factor (M-CSF) transcripts in rat bone marrow (22). With regard to trauma and injury, Sifri et al. (47) have shown that mesenteric lymph from male mice plays a direct role in bone marrow hematopoietic failure following trauma and hemorrhagic shock. Conversely, estrogen appears to selectively reduce a bone marrow cell subset,
which is highly enriched with functional lymphoid precursors (31). The aim of the present study was to elucidate whether sex and age impact bone marrow differentiation and function in a murine model of trauma and hemorrhagic shock.

MATERIAL AND METHODS

Animals. Inbred male and female C3H/HeN mice (Charles River Laboratories, Wilmington, MA), 21–27 days [8–14 g body weight (BW)], 6–8 wk (23–26 g BW), and 22–24 mo old (28–37 g BW; retired breeder) were used for this study. The animals were allowed to acclimate to the animal facility at the University of Alabama at Birmingham. The group-housed mature (6–8 wk) and aged (22–24 mo) females were able to synchronize their estrus cycle, which was determined by daily examination of the vaginal smear. In this study, mature and aged female mice in the proestrus stage, with elevated circulating estrogen levels, and premature females, without estrus cycle, were included. 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. This study was approved by the Institutional Animal Care and Use Committee of University of Alabama at Birmingham.

Experimental groups. Female and male mice, 21–27 days (premature), 6–8 wk (mature), or 22–24 mo old (aged), were randomized into one or two groups containing six to eight animals each. Group 1 consisted of sham-operated animals, and the animals in group 2 underwent the trauma-hemorrhage procedure. Twenty-four hours after the end of resuscitation or sham operation, the animals were killed by methoxyflurane overdose, and bone marrow was harvested aseptically.

Trauma-hemorrhage procedure. Male and female mice of all ages in the trauma-hemorrhage groups were lightly anesthetized with methoxyflurane (Metofane, Pitman-Moore, Mundelein, IL) and restrained in a supine position. A 2.5-cm midline laparotomy (i.e., soft tissue trauma) was performed, and the abdominal incision was then closed aseptically in two layers using 6–0 sutures (Ethilon, Ethicon, Somerville, NJ). Both femoral arteries were then cannulated and polyethylene 10 tubing (Clay-Adams, Parsippany, NJ) was used to implant a pressure transducer in the femoral arteries; however, neither hemorrhage nor fluid resuscitation was performed. The intact femoral arteries, which is highly enriched with functional lymphoid precursors (31). The aim of the present study was to elucidate whether sex and age impact bone marrow differentiation and function in a murine model of trauma and hemorrhagic shock.

Bone marrow preparation for flow cytometric analysis. All antibodies and isotype-specific controls conjugated to FITC, phycoerythrin (PE), allophycocyanin (APC), peridinin chlorophyll-a (PerCP), or biotin were purchased from PharMingen. The following antibodies and isotype-specific controls were used: 2B8 [rat (Wistar) IgG2a,α; anti-CD117(c-kit)-PE], RAM34 [rat IgG2a,α; anti-CD34-ITC], D7 [rat (Lewis) IgG2a,α; anti-Ly-6A/E(SCa-1)-biotin], 145–2C11 [Armenian Hamster IgG, group 1,κ; anti-CD3-PerCP], RA3–6B2 [rat IgG2a,α; anti-CD45R/B220-APC], M1/70 [rat (DA) IgG2b,α; anti-CD11b-APC], RB6–8C5 [rat IgG2a,α; anti-Ly-6G(GR-1)-APC], and streptavidin-PerCP. Bone marrow cells were resuspended in PBS containing 1% FCS and 0.1% sodium azide (complete PBS), blocked with a rat anti-mouse CD16/CD32 (FcγRIIa-II) antibody (1 μg/10^6 cells) for 5 min at 4°C and then labeled with the antibodies and isotype-specific controls (1 μg of antibody per 10^6 cells). Samples were incubated for 30 min on ice in the dark and thereafter washed once with complete PBS. For the second step, cells were incubated with streptavidin-PerCP (1:200) for 30 min in the dark at room temperature and washed twice with complete PBS. Samples were kept on ice in the dark, and all measurements were analyzed within 30 min after the staining procedure was completed. FITC, PE, APC, and PerCP were analyzed with a Becton-Dickinson FACS Calibur flow cytometer (San Jose, CA) fitted with a 488-nm argon laser and 635-nm red diode lasers. Filter settings were for FITC (530-nm-wide band-pass filter), PE (585-nm dichroic filter), APC (661-nm-wide band-pass filter), and PerCP (670-nm long-pass filter). After appropriate instrument settings and spectral compensations, the instrument settings were not changed, and stability was checked regularly. A minimum of 50,000 events were assessed using log-amplified fluorescence signals and linearly amplified side- and forward-scatter signals. PC-lysis version 1.0 software (Becton-Dickinson) was used to analyze the data.

Bone marrow cell culture preparation. For the proliferation assays, bone marrow cells were resuspended in complete media (RPMI-1640, 10% fetal bovine serum, penicillin G 50 U/ml and streptomycin 50 μg/ml) at a final concentration of 1 × 10^5 cells/ml and incubated in a 96-well microtiter plate containing 2 × 10^5 cells per well. The cell’s ability to proliferate in response to granulocyte M-CSF (GM-CSF) (Genzyme, Cambridge, MA) and IL-3 (Genzyme) alone or together with zero (negative control), 100 units/ml GM-CSF, or 100 units/ml IL-3 was determined by incubation for 48 h. Optimal IL-3 and GM-CSF concentrations were determined in preliminary experiments (data not shown). The extent of proliferation was measured using the [3H]thymidine incorporation technique, as described by Stephan et al. (49).

For LPS-stimulated bone marrow cultures, the cells were resuspended in complete media and stimulated with 10 μg/ml LPS (Sigma Chemical) and incubated for 24 h at 37°C, 5% CO₂, and 90% humidity. Cell-free supernatants were collected and stored at −80°C until assayed for cytokine production.

Assessment of cytokine and PGE₂ release. TNF-α, IL-6, and IL-10 levels were determined by ELISA (PharMingen), and plasma PGE₂ levels were measured by enzyme immunoassay (Cayman Chemical, Ann Arbor, MI). Plasma samples were purified by C-18 solid-phase extraction before assay to obviate sample-specific interferences. Plasma protein content was determined using the DC Protein Assay (Bio-Rad, Hercules, CA).

Statistics. Data are presented as the means ± SE of six to eight animals in each group. One-way ANOVA for multiple comparison followed by Tukey’s or Dunn’s test were employed to determine the significance of the differences between experimental means. P < 0.05 was considered significant for all statistical analysis.

RESULTS

Age- and sex-related changes in bone marrow differentiation after trauma-hemorrhage. In male and female sham animals, the same proportion of the pluripotent, predominantly stem cell-associated bone marrow cell subset Sca-
1+CD117+CD34−lin+/− was observed independent of the age (Fig. 1). The Sca-1+CD34−lin+/− bone marrow cell subsets, which are negative for CD117 containing hematopoietic stem cells, decreased only in aged male mice. After trauma-hemorrhage, both of these early pluripotent stem cell-associated bone marrow cell subsets increased in young mice of both sexes (Fig. 1). In the trauma-hemorrhage mature group, the stem cell subset Sca-1+CD34−lin+/−CD117+ increased only in proestrus females, but not in males. Aged animals of both sexes displayed unchanged stem cell-associated bone marrow cell subsets, but aged males had generally significantly lower amounts of the stem cell antigen-1 (Sca1)+ cells after trauma-hemorrhage than young male mice under such conditions (Fig. 1).

The amount of the pluripotent progenitor cell subsets, which are not expressing Sca1, but high CD117 and CD34, declined with aging, independent of sex in the sham groups (Fig. 2). After trauma-hemorrhage, the proportion of those cells remained unchanged in mature and aged mice of both sexes; however, trauma-hemorrhage induced a significant decline of

<table>
<thead>
<tr>
<th></th>
<th>Young Male</th>
<th>Young Female</th>
<th>Mature Male</th>
<th>Mature Female</th>
<th>Aged Male</th>
<th>Aged Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>1.2 ± 0.3</td>
<td>1.2 ± 0.2</td>
<td>1.9 ± 0.9</td>
<td>1.9 ± 0.8</td>
<td>0.9 ± 0.2</td>
<td>2.1 ± 0.4</td>
</tr>
<tr>
<td>c-Kit</td>
<td>5.3 ± 0.7</td>
<td>4.1 ± 0.7</td>
<td>4.8 ± 1.2</td>
<td>5.2 ± 1.1</td>
<td>1.5 ± 0.4</td>
<td>4.6 ± 1.3</td>
</tr>
<tr>
<td>Trauma-Hemorrhage</td>
<td>3.3 ± 0.4</td>
<td>3.9 ± 0.4*</td>
<td>2.9 ± 1.0</td>
<td>4.0 ± 0.4*</td>
<td>1.6 ± 0.3*</td>
<td>2.3 ± 0.3</td>
</tr>
</tbody>
</table>

Fig. 1. Flow cytometric analysis of whole bone marrow harvested 24 h after initiation of the experiment from young, mature, and aged mice, subjected to either sham procedure or trauma-hemorrhage (T-H). Cells were incubated with antibodies against anti-Sca-1(Ly-6A/E)-Sca1-peridinin chlorophyll-a (PerCP), anti-CD34-FITC, and anti-CD117(c-kit)-phycoerythrin (PE). A: representative dot plots of cells gated on the absence of CD34. x-Axis represents fluorescence intensity of Sca1 expression, and y-axes of CD117 expression. The top and the bottom right gates represent Sca1+CD34−lin+/− pluripotent stem cell-associated bone marrow cell subsets, either positive or negative for the expression of CD117. B: means representing Sca1+CD34−lin+/− pluripotent stem cell-associated bone marrow cell subsets, either positive or negative for the expression of CD117. a: young mice; b: mature mice; c: aged mice. Values are means ± SE of not less than 6 independent experiments. ANOVA: *P < 0.05 vs. sham; §P < 0.05 vs. young; #P < 0.05 vs. mature.
this pluripotent progenitor cell subset in young males and females (Fig. 2).

Age- and sex-related changes in the expression of lineage marker on murine bone marrow cells after trauma-hemorrhage. Differentiation of the bone marrow cells to cells that expressed lymphocyte (CD3, B220) or myeloid (Gr-1, CD11b) lineage markers showed a decrease in bone marrow cells expressing lineage marker for B- and T-lymphocytes in the sham groups with increased age (Table 1). Trauma-hemorrhage induced enhanced differentiation into myeloid progenitor cell subsets, which expressed CD11b+Gr-1+ in all groups (Table 1). In mature proestrus females, bone marrow cells also expressing both lymphoid cell lineage markers (CD3, B220) increased under those conditions. In mature and aged proestrus females, but not in males, trauma-hemorrhage led to a differentiation of the bone marrow cells toward a monocyte/macrophage (CD11b+Gr-1+) lineage (Fig. 3).

Alterations of bone marrow proliferation in response to IL-3 and GM-CSF. The proliferative response of bone marrow cells to IL-3 and GM-CSF was comparable in all sham groups, irrespective of sex and age. Although early pluripotent stem cell-associated bone marrow cells increased in the young group and mature proestrus females after trauma-hemorrhage, stimulation with IL-3 did not enhance proliferative responses of

Fig. 2. Flow cytometric analysis of whole bone marrow harvested 24 h after initiation of the experiment from young, mature, and aged mice, subjected to either sham procedure or T-H. Cells were incubated with antibodies against anti-Sca-1(Ly-6A/E)-Sca1-PerCP, anti-CD34-FITC, and anti-CD117(c-kit)-PE. A: representative dot blots of cells gated on the absence of Sca1. x-Axis represent fluorescence intensity of CD34 expression, and y-axis of CD117 expression. The gate was set on cell subset containing cells that are Sca1−CD34−CD117−lin−/−, representing progenitor cell-associated bone marrow cells. B: means representing cells that are gated on Sca1−CD34−CD117−lin−/−, displaying progenitor cell-associated bone marrow cells. a: young mice; b: mature mice; c: aged mice. Values are means ± SE of not less than 6 independent experiments. ANOVA: *P < 0.05 vs. sham; §P < 0.05 vs. young.
those cells compared with the sham groups (Fig. 4). In young animals, the observed trauma-hemorrhage-induced decline in pluripotent progenitor cells was paralleled with a decreased proliferative response to GM-CSF (Fig. 4). Similar alteration in GM-CSF-induced bone marrow proliferation was evident in mature males following trauma-hemorrhage, whereas proliferation increased in aged proestrus female mice (Fig. 4). While bone marrow cells from mature males showed depressed proliferative response after stimulation with both IL-3 and GM-CSF following trauma and hemorrhage, no effects were seen in young and aged mice of both sexes (Fig. 4).

Productive capacity of whole bone marrow for the monocyte/macrophage-related mediators. In general, bone marrow cells of young mice from the sham groups produced lower amounts of TNF-α, whereas the release of IL-6 was greater in

**Table 1. Flow cytometric analysis of whole bone marrow from sham and trauma-hemorrhage mice (% of whole population)**

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Mature</th>
<th>Aged</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>CD33&lt;sup&gt;+&lt;/sup&gt;</td>
<td>19.4±3.2</td>
<td>20.9±1.4</td>
<td>18.1±1.1</td>
</tr>
<tr>
<td>B20&lt;sup&gt;+&lt;/sup&gt;</td>
<td>28.6±3.9</td>
<td>30.0±1.8</td>
<td>31.0±3.7</td>
</tr>
<tr>
<td>CD11b&lt;sup&gt;+&lt;/sup&gt;Gr-1&lt;sup&gt;+&lt;/sup&gt;</td>
<td>41.9±2.8</td>
<td>60.0±4.6&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>49.4±1.5</td>
</tr>
<tr>
<td>CD11b&lt;sup&gt;+&lt;/sup&gt;Gr-1&lt;sup&gt;-&lt;/sup&gt;</td>
<td>7.7±1.3</td>
<td>8.1±1.4</td>
<td>7.8±1.0</td>
</tr>
<tr>
<td>CD11b&lt;sup&gt;-&lt;/sup&gt;Gr-1&lt;sup&gt;-&lt;/sup&gt;</td>
<td>4.9±1.2</td>
<td>4.6±0.8</td>
<td>3.1±0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE in % of whole bone marrow cells; n = 6–8/group. Flow cytometric analysis is of whole bone marrow harvested at 24 h after initiation of the experiment from young, mature, and aged mice after sham operation or trauma-hemorrhage (T-H). Cells were incubated with antibodies against anti-CD3-peridinin chlorophyll-α (PerCP) and anti-CD45R/B220-allophycocyanin (APC) or anti-CD11b-PerCP and anti-GR-1 (Ly-6G)-APC. Data are presented of bone marrow cells that were positive for staining with antibodies to selected markers described above. ANOVA: *P < 0.05 vs. sham. †P < 0.05 vs. sham/young.
response to LPS compared with the mature and aged groups, independent of sex (Figs. 5 and 6). These effects were reversed with increased age. Following trauma-hemorrhage, the cytokine productive capacities for TNF-α and IL-6 increased in young mice of both sexes; however, this trend was not statistically significant (Figs. 5 and 6). In the mature and aged groups, trauma-hemorrhage induced a decreased release of TNF-α in males but not in proestrus females (Figs. 5 and 6). Similar alteration occurred for IL-6 in mature males, but remained unchanged in the aged groups after trauma-hemorrhage (Figs. 5 and 6).

LPS-induced release of the anti-inflammatory mediators IL-10 and PGE₂ was comparable in all sham groups (Figs. 7 and 8). In young mice, trauma-hemorrhage had no effect on the release of either mediator. In contrast, the production of IL-10 and PGE₂ was significantly increased in mature and aged males, whereas their release was unchanged in proestrus females following trauma-hemorrhage.

**DISCUSSION**

With respect to the number of residing immune cells, the bone marrow represents the largest immunocompetent organ in the body. Since trauma is associated with increased need and consumption of immune cells, increased differentiation and allocation of these cells from the bone marrow after injury would be beneficial to the host. Previous studies indicated that trauma alters the bone marrow myelopoietic response’s proliferative capacities (15, 25, 26, 35, 41). Our present results indicate that bone marrow differentiation and responses after trauma-hemorrhage are age and sex dependent. The proportion of pluripotent stem cell-associated bone marrow cell subsets did not change with age, but the amount of pluripotent progenitor cell subsets declined in the mature and aged groups, independent of sex. Following trauma-hemorrhage, proestrus females, which have elevated circulating estrogen levels, demonstrated an increased differentiation of bone marrow cells.

![Fig. 5](image_url) Release of TNF-α by whole bone marrow harvested 24 h after initiation of the experiment, in presence of 10 μg/ml LPS. A: young mice; B: mature mice; C: aged mice. Cytokine levels were determined by a specific ELISA. Values are means ± SE; n = 6–8/group. ANOVA, *P < 0.05 vs. sham.

![Fig. 6](image_url) Release of IL-6 by whole bone marrow harvested 24 h after initiation of the experiment, in presence of 10 μg/ml LPS. Cytokine levels were determined by a specific ELISA. A: young mice; B: mature mice; C: aged mice. Values are means ± SE; n = 6–8/group. ANOVA, *P < 0.05 vs. sham.

![Fig. 7](image_url) Release of IL-10 by whole bone marrow harvested 24 h after initiation of the experiment, in presence of 10 μg/ml LPS. Cytokine levels were determined by a specific ELISA. A: young mice; B: mature mice; C: aged mice. Values are means ± SE; n = 6–8/group. ANOVA, *P < 0.05 vs. sham.
Bone marrow cell subsets Sca1 (18). In the sham groups, the pluripotent, stem cell-associated bone marrow cell subsets are characterized by expression of Sca1, c-kit (18). These predominantly stem cell-associated bone marrow cell subsets are reversibly modulated by 10.220.33.4 on October 14, 2017 http://jap.physiology.org/ Downloaded from

towards monocyte/macrophage lineage cells, independent of age, which was associated with maintained bone marrow immune response.

The hematopoietic system is organized as a hierarchy of clonogenic cell types, with differing capacities for self-renewal, proliferation, and differentiation (18). Early murine pluripotent stem cells (hematopoietic stem cells) represent <0.1% of the stem cell-associated bone marrow cell subsets (18). These predominantly stem cell-associated bone marrow cell subsets are characterized by expression of Sca1, c-kit (CD117) at low levels, and they are either negative or positive for CD34 (50). They are normally negative for the lineage markers expressed on terminally differentiated lymphocytes (CD3, B220), myeloid (Gr-1, CD11b), and erythroid (TER-119) cells (18). However, dependent on the activation state, CD117+/−, CD34+/−, and lin+/− are reversibly modulated (18). In the sham groups, the pluripotent, stem cell-associated bone marrow cell subsets Sca1+CD34−CD117+lin+/− were unchanged throughout all age cohorts in our study. Stability in bone marrow cellularity has also been demonstrated in humans (12). A subgroup, which was negative or had low expression of CD117 normally expressed by early pluripotent stem cells, declined in aged males, but not in aged proestrus females. Hematopoietic stress (i.e., trauma-hemorrhage) caused a significant increase in the stem cell-associated Sca1+ bone marrow cell subsets in young animals. This ability of early bone marrow cell subsets in young individuals to rapidly expand may represent one reason for the rapid hematopoietic recovery in children following hematopoietic stress (52). In the mature group, only proestrus females exhibited comparable alterations under such conditions. Aged males and females demonstrated unchanged Sca1 positive bone marrow cell subsets, but this population was significantly decreased in aged males compared the young individuals. It is known that androgen and estrogen levels decrease with age, leading to a decrease in bone mass that is reversible with steroid replacements (3). Despite age-dependent changes in the hematopoietic stem and progenitor cell compartment (38), we can postulate that female sex hormones play a crucial role in females with respect to our observations.

Under stressful conditions, hematopoietic stem cells are recruited into active hematopoiesis and undergo a series of maturational cell divisions, during which time progeny of self-renewing divisions can coexist with various classes of progenitor cells, with progressively restricted proliferation and differentiation potentials (18, 48). In particular, early progenitor cells represent the cell subset that differentiates into myeloid, lymphoid, and erythroid precursors. These cells are characterized by expression of CD34, high CD117, and neither or low expression of Sca1 (18). In the present study, early progenitor cells decreased with age. Under stressful conditions, fewer precursors may be available to further differentiate and proliferate and may represent one reason for the restricted bone marrow response seen in aged individuals under such conditions (38).

Aging is associated with major changes in hematopoietic stem/progenitor cells and a decline in lymphoid lineage, and very early B-lineage precursors has been observed (20, 33). Our results demonstrate a decreased numbers of CD3+ or B220+ lymphoid precursors in the mature and aged groups. Trauma-hemorrhage, however, increased lymphoid precursors in mature proestrus females. Earlier studies indicate that estrogen is a negative regulator of the early stage of lymphopoiesis (21, 31), whereas differentiated lymphocytes are resistant to the effects of estrogen (55). Therefore, the increased amount of lymphoid cells in proestrus females may represent more differentiated lymphoid precursors. These cells may be released into circulation and migrate to the reproductive tract during the diestrus stage of the estrus cycle (27). With aging, this effect seems to be abrogated due to age-dependent alterations in the lymphoid compartment (20, 24, 33, 34).

Trauma injury and infection have been shown to be associated with an increase in myeloid progenitor cells in the circulation and increased production of granulocyte/macrophage colony-forming units (35, 46). Our results also indicated that the amount of granulocyte/macrophage progenitor cells CD11b+Gr-1+ increased in the bone marrow after trauma-hemorrhage in proestrus and aged females. Traumatic injury increases the expression of M-CSF receptor in myeloid progenitor cells, which represents the most important stimulator for this shift toward monocytosis (41). One explanation for the unchanged monocyte/macrophage precursors in traumatized males may be the observation that M-CSF transcripts are suppressed by androgens (22). Although early studies indicated that ovariectomy augments generation of monocyte/macrophage precursors (10), our results indicate that trauma-hemorrhage was associated with differentiation of bone marrow cells toward monocyte/macrophage lineage cells in proestrus females, but not in males.

Trauma-hemorrhage causes a bone marrow depression associated with a decrease in the proliferation of granulocyte/macrophage colony-forming units (25). The differentiation of myeloid lineage-restricted progenitor cells is initially con-
trolled by IL-3, which matures the progenitors to a point where they can respond to specific lineage-restricted glycoproteins (9). In particular, GM-CSF matures monocytes, but is also capable of producing granulocytes from bipotential progenitor cells (9). We observed that IL-3-dependent proliferative capacities remained unchanged after trauma-hemorrhage. In contrast, stimulation with GM-CSF revealed a depressed proliferation in young mice and mature male mice, but not in proestrus females under such conditions. Costimulation with IL-3 blunted this depression in young mice, but not in mature males. Previous studies have shown that T cells from proestrus females produce greater amounts of IL-3 in response to LPS compared with males (2). Since administration of IL-3 receptor agonist has been demonstrated to stimulate multilinage hematoepoietic recovery, it can be speculated that IL-3 may protect against trauma-induced bone marrow alterations (28). This effect is evident in young mice, since depressed responses of granulocyte/macrophage progenitor cells were reversed after costimulation with IL-3. Thus trauma-induced bone marrow defects are not at the levels of early myeloid cells, but rather at the level of granulocyte/macrophage progenitor cells. In contrast, in mature male mice, the suppressive effects of androgen abolished IL-3 costimulatory action with GM-CSF. This was not evident in aged male mice with decreased levels of circulating androgens. The importance of androgen’s action under such conditions was further evidenced that the depressed proliferation of granulocyte/macrophage progenitor cells was not observed in aged males. The expression of the GM-CSF receptor declines after trauma in monocytes (8), which may contribute to the depression of granulocyte/macrophage proliferation in young and mature males.

In males, trauma-hemorrhage impairs monocyte/macrophage functions, as indicated by the decreased proinflammatory cytokine release in vitro and increased release of mediators which downregulate host immune responses (2, 7). Proestrus females are protected against trauma-induced immunodepression (2, 7). Although the exact mechanisms by which sex steroids influence immune cells remain unclear, the response is likely direct, since sex steroid receptors have been identified on T cells and macrophages (40, 56). Our results revealed that LPS-stimulated cytokine productive capacity of bone marrow cells exhibited similar sex-dependent alterations following trauma-hemorrhage as demonstrated for peripheral monocyte/macrophages. However, in young mice, the trauma-induced changes in cytokine release were subtle and sex independent. In general, we observed that bone marrow capacities to produce IL-6 declined with age, whereas productive capacities for TNF-α increased with age. These divergent changes may be explained by the fact that TNF-α infusion is capable of downregulating IL-6 release in vitro after LPS stimulation (37). This increased release of IL-6 may also play an important role in the increased proliferative capacities of bone marrow cells of young individuals, since studies indicate that IL-6 potentiates GM-CSF and IL-3-mediated bone marrow formation (17).

IL-10 and PGE2 are counterregulatory mediators in host immune responses (11, 44, 45). IL-10 has been demonstrated to suppress severe acute graft vs. host disease after stem cell transplantation and downregulate the inflammatory response (39). On the other hand, results show that IL-10 increases Bcl-2 expression and survival of human hematopoietic progenitor cells that are committed to the myeloid lineage (53). Increased bone marrow IL-10 release may downregulate inflammatory responses, since it was paralleled by decreased inflammatory mediator release. PGE2 has been demonstrated to be, at least in part, responsible for bone marrow depression in burn-injected mice and is a key mediator in the gram-negative sepsis-induced macrophage suppression of granulocyte and macrophage maturation (13, 14). In the present study, we observed increased release of PGE2 in traumatized mature and aged male mice in parallel with depressed proliferation and inflammatory mediator release after in vitro stimulation with LPS.

In summary, these results indicate for the first time that sex- and age-specific effects take place in bone marrow differentiation and immune responses after trauma-hemorrhage. Females in the proestrus state of the estrus cycle, with high levels of estrogen, demonstrated maintained bone marrow immune response compared with males following trauma-hemorrhage, independent of age. Therefore, the suppressive effects of male sex steroids may take place in early differentiated immune cells.

GRANTS

This work was support by National Institutes of Health (NIH) Grant R01 GM37127. M. G. Schwacha is in part supported by NIH Grant K02 AI049960. Present address of C. P. Schneider: Department of Surgery, Klinikum Grosshadern, Ludwig-Maximilians-University, Munich, Germany.

REFERENCES