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Short Communication

Sex-specific impact of psychosocial stress on hematopoiesis and blood leukocytes

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Stress exposure has been shown to modulate innate and adaptive immune responses. Indeed, stress favors myelopoiesis and monocyte generation and contributes to cardiovascular disease development. As sex hormones regulate innate and adaptive immune responses, we decided to investigate whether stress exposure leads to a different immune response in female and male mice. Our data demonstrated that psychosocial stress-induced neutrophilia in male, but not female mice. Importantly, we identified that B-cell numbers were reduced in female, but not male mice upon exposure to stress. Thus, our study revealed that the stress-induced immune alterations are sex-dependent, and this is an important feature to consider for future investigations.

Keywords: B cells · Neutrophils · Sexual dimorphism · Stress



Additional supporting information may be found online in the Supporting Information section at the end of the article.

Introduction

Previous studies established a critical role of psychosocial stress (PS) on innate immunity [1–3]. A pioneering report revealed that stress increased the number of Ly6C^{high}, but not Ly6C^{low} blood monocytes, as well as neutrophils [1]. PS promoted bone marrow (BM) myelopoiesis resulting in augmented hematopoietic stem cells, monocyte, and neutrophil numbers in stressed mice [1]. Furthermore, PS induced norepinephrine release, which by acting on the sympathetic nervous system signaling in the BM triggered a decrease in CXCL12 expression [1]. CXCL12 reduc-

tion resulted in decreased BM monocyte and neutrophil retention and favored their release into the blood. Mechanistically, blocking B3-adrenergic receptors restored BM CXCL12 expression and decreased blood monocyte and neutrophil numbers in stressed mice, highlighting the essential role of this axis during PS-induced immune cell modulations [1]. Recently, the impact of PS on monocytes was directly assessed. PS induced BM progenitor accumulation, while CXCL12 levels were similar between control and stressed mice [2]. In contrast to the initial report, similar monocyte and neutrophil numbers were present in blood in control and PS-exposed animals [2]. Yet, PS induced epigenetic reprogramming in monocytes that translated into a pro-inflammatory phenotype associated with metabolic rewiring [2].

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Bastien Dolfi and Alexandre Gallerand contributed equally.

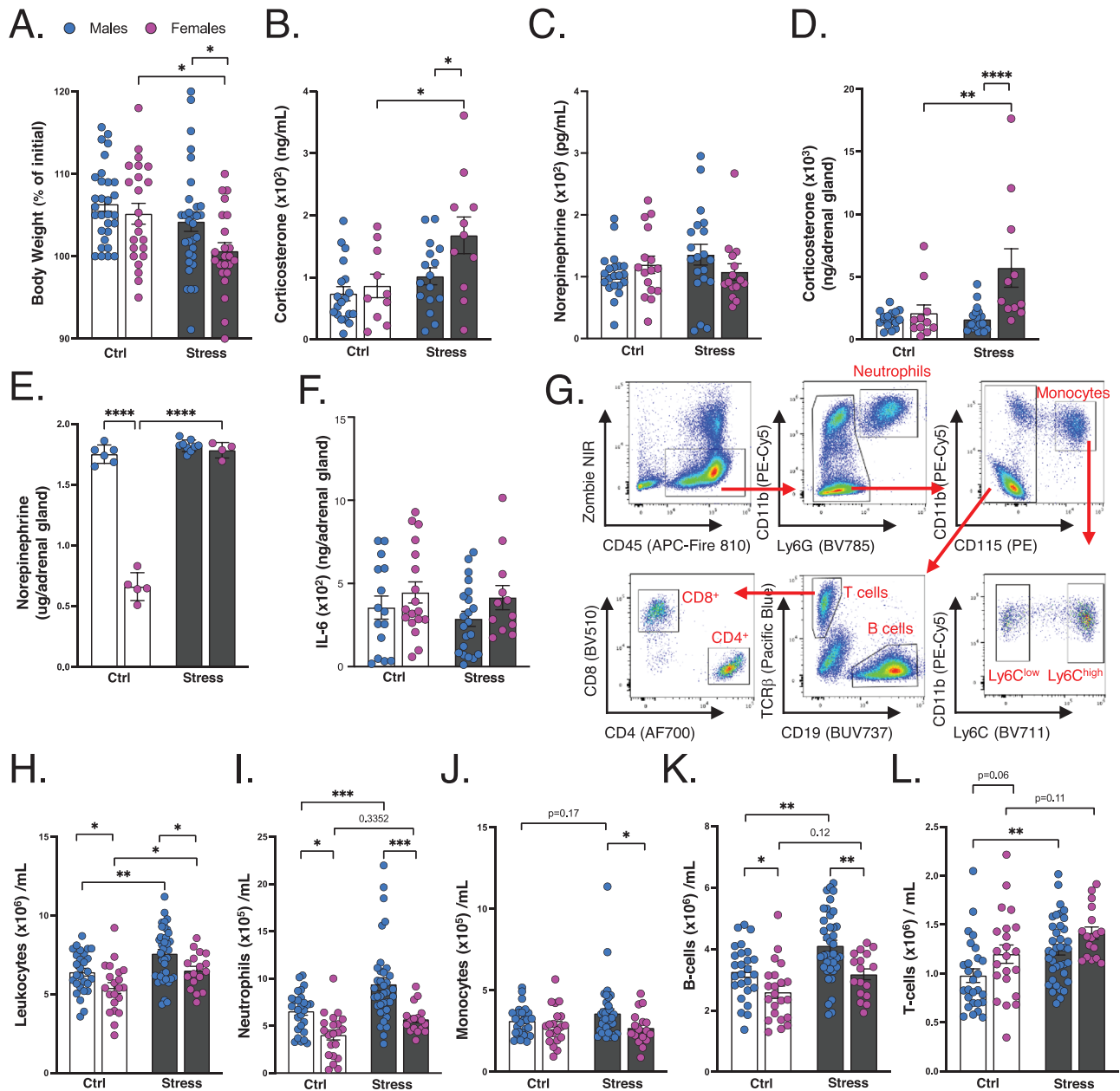


Figure 1. Stress-induced hormone and blood immune cell modification in a sex-dimorphic manner. (A) Percentage of weight gain in control (males $n = 30$, females $n = 23$) and stressed (males $n = 31$, females $n = 23$) animals. (B) Plasma corticosterone concentration in control (males $n = 19$, females $n = 10$) and stressed (males $n = 16$, females $n = 11$) mice. (C) Plasma norepinephrine concentration in control (males $n = 21$, females $n = 16$) and stressed (males $n = 20$, females $n = 15$) mice. (D) Adrenal gland corticosterone concentration in control (males $n = 17$, females $n = 11$) and stressed (males $n = 20$, females $n = 11$) mice. (E) Adrenal gland norepinephrine concentration in control (males $n = 6$, females $n = 5$) and stressed (males $n = 9$, females $n = 4$) mice. (F) Adrenal gland IL-6 concentration in control (males $n = 15$, females $n = 18$) and stressed (males $n = 21$, females $n = 12$) mice. (G) Gating strategy to identify blood immune cells. (H) Quantification of blood CD45⁺ leukocytes in control (males $n = 28$, females $n = 21$) and stressed (males $n = 38$, females $n = 17$) mice. (I) Quantification of blood neutrophils in control (males $n = 27$, females $n = 21$) and stressed (males $n = 37$, females $n = 17$) mice. (J) Quantification of blood monocytes in control (males $n = 28$, females $n = 21$) and stressed (males $n = 38$, females $n = 17$) mice. (K) Quantification of blood B cells in control (males $n = 28$, females $n = 21$) and stressed (males $n = 38$, females $n = 17$) mice. (L) Quantification of blood T cells in control (males $n = 27$, females $n = 22$), and stressed (males $n = 38$, females $n = 17$) mice. All data are presented as means \pm SEM. Blue dots indicate male and pink dots female mice. Panels B to F represent three independent experiments. Panels H to L represent five independent experiments. Two-way ANOVA multiple comparisons were used to determine statistical significance. ns $p > 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.001$.

Previous studies documented sex-related changes in the immune response [4–7]. A sex dimorphism was documented in adrenal gland macrophages, serous cavity macrophages [8–11], and adipose tissue regulatory T cells [12]. However, whether PS modulations of immune cell populations are affected by sex dimorphism is yet to be established since the pioneering studies were performed solely in female mice [1], [2]. In the present study, we explored the impact of PS on hematopoiesis and blood-immune cell composition in male and female mice. In female mice, PS resulted in a hormonal response characterized by increased production of corticosterone and altered B-cell lymphopoiesis, although circulating B cells were not affected. In male mice, PS exposure resulted in systemic neutrophilia but did not impact corticosterone levels. Orchidectomy prevented neutrophilia in stressed animals and promoted lymphopoiesis independently of stress.

Results

We submitted C57BL/6JRj female and male animals to a previously established and validated PS protocol [1]. Mice were stressed for 3 weeks (chronic stress). Female mice exposed to stress failed to gain weight during the procedure, while stress did not impact weight in male animals (Fig. 1A). We next measured stress hormone concentrations in plasma. Blood corticosterone concentration was increased in stressed female, but not male mice (Fig. 1B), while NE concentrations remained similar in both sexes (Fig. 1C). Thus, a sex-related difference in stress hormone release in plasma appeared upon stress exposure. We then aimed to determine whether these differences could be resulting from altered production in adrenal glands. In line with our observations in plasma, stressed females, but not males, displayed increased corticosterone concentrations in adrenal glands (Fig. 1D). Adrenal gland NE content was about threefold lower in unstressed females than in males but increased in stressed females while remaining unchanged in males (Fig. 1E). The concentration of the stress-induced cytokine IL-6 in adrenal glands was similar in PS-exposed and control female and male mice suggesting no impact of stress on inflammation (Fig. 1F). Taken together, these data suggested a higher adrenal production of stress hormones upon stress exposure in female mice leading to increased systemic levels, while this was not the case in male mice.

We next investigated whether the differential hormonal response to PS could be linked to blood immune cell content. Flow cytometry analysis of blood CD45⁺ immune cells (Fig. 1G) showed that stress increased the number of white blood cells (WBC) in both sexes (Fig. 1H). Exposition to PS triggered neutrophilia in male, but not female mice (Fig. 1I and Fig. S1A). Monocyte numbers were not significantly increased by stress exposure (Fig. 1J). Of interest, Ly6C^{low} monocytes tended to increase selectively in male mice, while Ly6C^{high} monocyte numbers remained unchanged (Fig. S1B and C).

We next observed that, in basal conditions, B-cell numbers were higher in male animals compared with females and were

further increased by stress (Fig. 1K and Fig. S1D). In contrast with B cells, blood T cells were more numerous in females compared with male animals but increased only in stressed males (Fig. 1L and Fig. S1E). Closer examination of T cells revealed that CD8⁺ T cells increased in both female and male mice upon PS exposure while CD4⁺ T cells were not significantly affected (Fig. S1F). Exposition to PS was thus marked by increased neutrophil and lymphocyte numbers in male mice. Although stressed female mice also showed increased total blood leukocyte numbers, the only subset that was specifically affected appeared to be CD8⁺ T cells.

We next hypothesized that the blood immune profiles we observed could reflect alterations of bone marrow hematopoiesis. Using spectral flow cytometry, we analyzed BM progenitor and mature immune cell content (Fig. 2A). We observed higher LSK (Lin⁻ Sca1⁺ cKit⁺) numbers in male animals in comparison to females with no impact of PS on LSK numbers (Fig. 2B). Stress exposure did not modulate long-term (LT) and short-term (ST) hematopoietic stem cells (Fig. 2C). Myeloid cell progenitor cells were also more numerous in male mice with higher numbers of common myeloid progenitors, granulocyte-monocyte progenitors, granulocyte progenitors (GPs), common monocyte progenitors, and monocyte-dendritic cell progenitors; however, those cells were unaffected by PS (Fig. 2D). We also observed higher BM monocyte numbers in male animals but did not detect PS-related modulations (Fig. 2E). The only clear effect of PS on hematopoiesis was restricted to female mice in which increased GP and monocyte-dendritic cell progenitor numbers were observed (Fig. 2D). Although we did not detect altered granulopoiesis in stressed male mice, neutrophils were more numerous in the bone marrow of those mice similar to what we observed in blood (Fig. 2F). Whether these phenotypes reflect increased longevity of neutrophils during stress or an accelerated flux in granulopoiesis remains to be determined.

Stress exposure augmented blood B-cell numbers and we investigated B-cell BM generation. Pre-pro B-cell precursors and pre-B cells were more numerous in females compared with male mice at steady state (Fig. 2G). PS decreased B-cell precursors in both sexes with a higher effect on females (Fig. 2G). We observed higher B-cell proportions and counts in female mice, in unison with more intense B-cell precursor generation and differentiation (Fig. 2H). Importantly, bone marrow B-cell numbers were negatively impacted by stress in female mice, but not in males despite our observation that stress could reduce the numbers of B-cell progenitors in both sexes (Fig. 2H). Finally, we asked whether antibody production could be impacted in a similar way. Plasma cell numbers were higher in the bone marrow and spleen of females compared with males at steady state; however, those differences were abrogated in stressed animals (Fig. 2I). These effects directly impacted plasma IgM and IgG titers, which were regulated in a similar way (Fig. 2J). While IgM levels tended to diminish in stressed females compared with their steady-state counterparts, IgG levels were significantly reduced in those mice (Fig. 2J). Overall, we observed that BM immune cell composition was

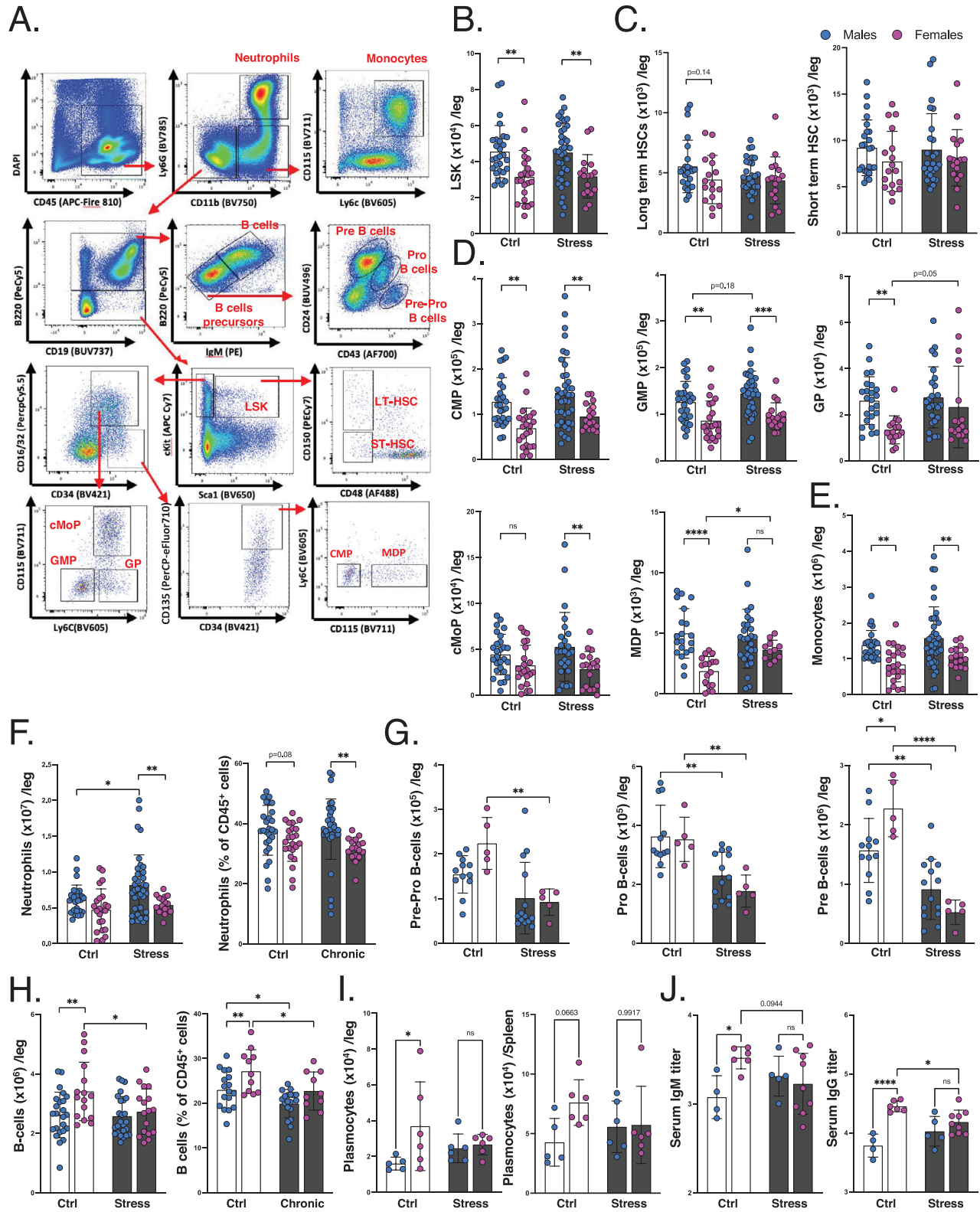


Figure 2. Sex-dimorphic bone marrow hematopoiesis and stress-induced changes. (A) Gating strategy to identify hematopoietic progenitors and mature cells in bone marrow. (B) Quantification of bone-marrow LSK cells ($\text{Ly6G}^- \text{CD11b}^- \text{B220}^- \text{cKit}^+ \text{Sca1}^+$) in control (males $n = 28$, females $n = 23$) and stressed (males $n = 38$, females $n = 17$) mice. (C) Quantification of bone-marrow long-term HSCs (control: males $n = 24$, females $n = 17$; stressed: males $n = 26$, females $n = 17$) and short-term HSCs (control: males $n = 24$, females $n = 17$; stressed: males $n = 25$, females $n = 17$). (D) Quantification of myeloid progenitor cells ($\text{Ly6G}^- \text{CD11b}^- \text{B220}^- \text{cKit}^+ \text{Sca1}^+$) in control and stressed mice. Common myeloid progenitors

different between female and male mice and PS exerted sex-dependent effects. Neutrophils and monocytes were more numerous in males while females had more B-cell precursors, B cells, and plasma cells. Importantly, stress negatively impacted antibody production in females and supported the expansion of neutrophils in males.

The spleen is a major reservoir for immune cells and could contribute to their generation [13]. Flow cytometry analysis (Fig. S2A) showed that PS-exposed animals have fewer immune cells in their spleen (Fig. S2B) which is associated with lower T-cell content but not B cells (Fig. S2C). Splenic neutrophil numbers remained similar upon PS exposure (Fig. S2D). At steady-state, monocyte numbers were similar between female and male mice (Fig. S2E). However, PS triggered increased splenic monocyte counts in males but not in females (Fig. S2E). Of note, stress tended to support IL-4 production by splenic CD4⁺ T cells in male but not female mice (Fig. S2F). Furthermore, T cells in females produced more IL-17A in comparison to male mice (Fig. S2F). Thus, the elevation of blood T cells by stress exposure could be a consequence of their release from the spleen.

We next sought to investigate the role of male sex hormones on blood and BM immune cell content. For this purpose, male mice underwent a mock surgery (Sham) or ablation of testes (orchidectomy, OCX) and were later submitted to the chronic stress procedure. OCX is well known to impact on thymus and overall T-cell compartment [14], [15]. We thus investigated thymus T-cell progenitor content in control and OCX mice (Fig. S3A). Castrated animals were characterized by higher numbers of T-cell progenitors. CD4/CD8 double-positive precursors were increased in OCX mice in comparison to controls (Fig. S3B). Double-negative T-cell precursors were also increased in OCX mice (Fig. S3C). This translated into increased numbers of CD4⁺ T cells and CD8⁺ T cells (Fig. S3D). Importantly, OCX animals exposed to PS presented fewer thymic immune cells compared with control mice (Fig. S3B–D) and altered IFN γ production in splenic T cells (Fig. S3E). Proportions of regulatory T cells were not modulated in the spleen (Fig. S3F). Thus, androgen production in males appeared as a major negative regulator of T-cell thymic generation and their release into blood upon stress exposure.

We observed that OCX triggered dynamic changes in blood immune cell populations at steady state, characterized by an increase of B-cell and a decrease of neutrophil frequencies

(Fig. 3A). Of note, Ly6C^{low} cells appear more predominant among monocytes in OCX mice at steady state (Fig. S3G). OCX mice had increased blood immune cell content that was not amplified by PS (Fig. 3B). Importantly, OCX mice did not present increased blood neutrophil counts when exposed to PS (Fig. 3C). This result suggests that stress-induced neutrophilia directly depends on male hormones. We then tested whether this could be linked to a medullary phenomenon. OCX males showed increased LSK and common myeloid progenitor numbers when exposed to PS, yet granulocyte-monocyte progenitor numbers were unaffected (Fig. S3H–I). We did not detect the regulation of GP numbers by hormones or stress. However, common monocyte progenitor numbers increased in stressed OCX males (Fig. 3D). Interestingly, Ly6C^{high} blood monocyte numbers tended to diminish while Ly6C^{low} monocyte numbers increased in stress OCX males (Fig. 3E). Together, these results suggest that male hormones could regulate monocyte generation and conversion to the Ly6C^{low} subset in blood in conditions of stress. Another striking observation was the increase of B-cell numbers in blood and bone marrow in OCX mice, independently of stress (Fig. 3F). Similarly, a number of medullary B-cell precursors were increased in those mice (Fig. 3G and Fig. S3J). These results are reminiscent of our observation that the B-cell compartment is more abundant in females compared with male mice at steady state and suggest that male hormones might be repressing B-cell lymphopoiesis. However, plasma IgM and IgG levels did not follow the female pattern in stressed OCX males (Fig. 3H). Instead, IgG production was increased in stressed OCX males while IgM levels appeared unregulated (Fig. 3H). Together, these results indicate that male hormones favor the onset of neutrophilia while they restrain B-cell lymphopoiesis and antibody production in stress conditions.

Conclusion

We observed that PS induced a well-defined stress hormone and immune cell signature in blood, BM, spleen, and thymus. We observed a sex-dimorphic immune response to stress which confirmed that studies should consider sex as a major determinant. We found that lymphocytes (T cells and B cells) were the main cell types increased in PS-exposed animals. While myelopoiesis was increased in males, B-cell BM generation was higher in females

(CMP): control (males $n = 27$, females $n = 23$) and stressed (males $n = 38$, females $n = 17$); granulocyte-monocyte progenitors (GMPs): control (males $n = 28$, females $n = 23$) and stressed (males $n = 37$, females $n = 17$); GP: control (males $n = 24$, females $n = 16$), and stressed (males $n = 25$, females $n = 17$); common monocyte progenitors (cMoPs): control (males $n = 27$, females $n = 22$), and stressed (males $n = 37$, females $n = 17$); monocyte-dendritic cell progenitors (MDP): control (males $n = 20$, females $n = 17$), and stressed (males $n = 28$, females $n = 11$). (E) Quantification of bone-marrow monocytes in control (males $n = 27$, females $n = 23$) and stressed (males $n = 37$, females $n = 17$) mice. (F) Quantification of bone-marrow neutrophils in control (males $n = 27$, females $n = 23$) and stressed (males $n = 37$, females $n = 17$) mice. (G) Quantification of bone-marrow B-cell progenitors in control (males $n = 12$, females $n = 5$) and stressed (males $n = 14$, females $n = 5$) mice. (H) Quantification of bone-marrow B cells in control (males $n = 24$, females $n = 16$) and stressed (males $n = 25$, females $n = 17$) mice. (I) Quantification of bone-marrow plasmocytes in control (males $n = 5$, females $n = 6$) and stressed (males $n = 6$, females $n = 6$) mice on the left. Quantification of splenic plasmocytes (males $n = 5$, females $n = 6$) and stressed (males $n = 6$, females $n = 6$) mice in the right. (J) Serum IgM titer (on the left) and IgG titer (on the right) in control (males $n = 5$, females $n = 6$) and stressed (males $n = 6$, females $n = 6$) mice. All data are presented as means \pm SEM. Blue dots indicate male and pink dots female mice. Panels B, D (CMP, GMP, cMoP), E and F represent five independent experiments. Panels B, D (GP and MDP), and H represent five independent experiments. Panel G represents two independent experiments, panels I and J represent one experiment. Two-way ANOVA multiple comparisons were used to determine statistical significance. ns $p > 0.05$; * $p < 0.05$; ** $p < 0.01$; **** $p < 0.001$.

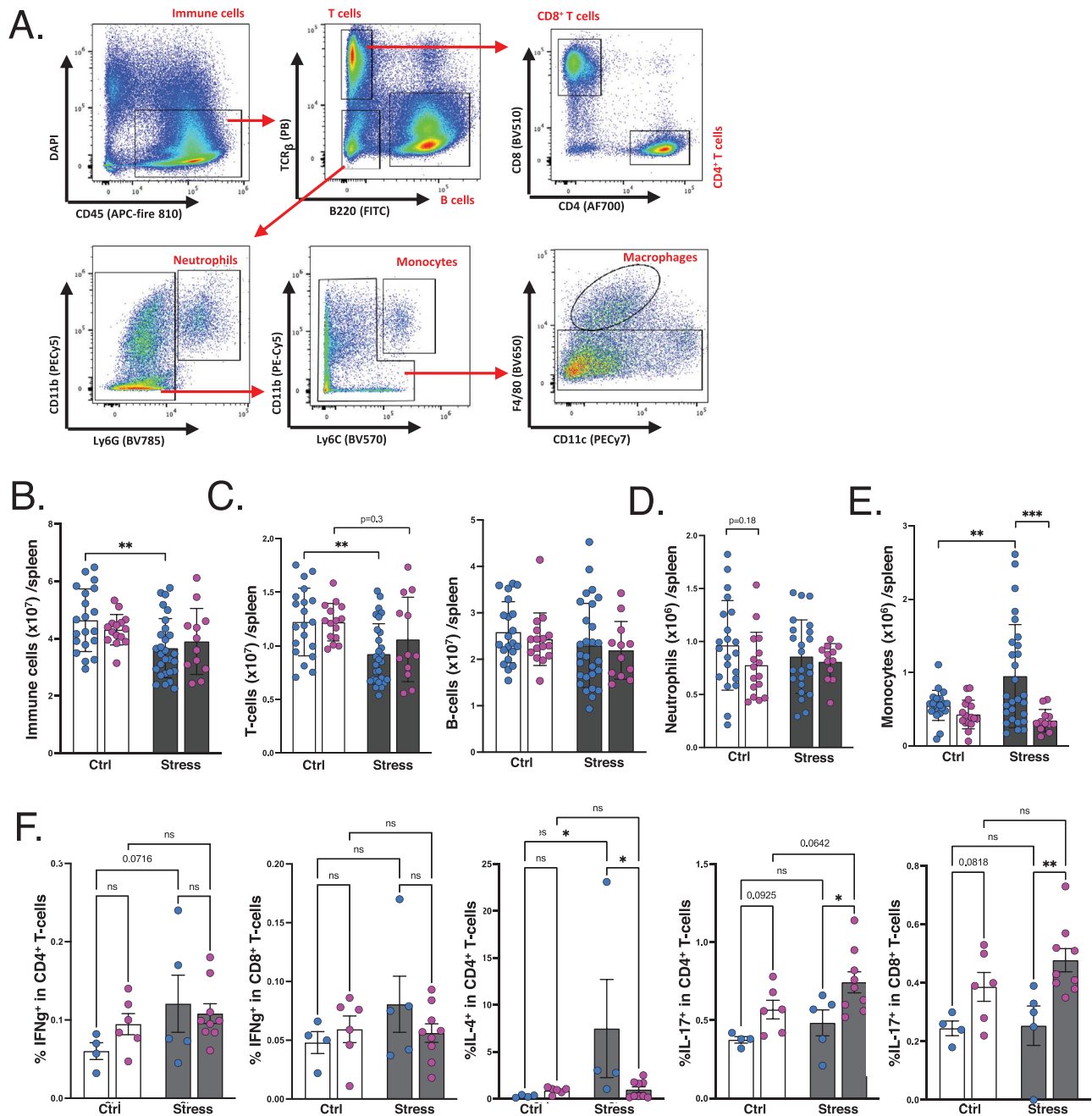


Figure 3. Impact of male hormones on immune cell generation at steady-state and upon stress exposure. (A) Immune cell frequencies in the blood of control and orchietomized (OCX) males. (B) Quantification of blood CD45⁺ immune cells in control (Ctrl and Sham $n = 10$, OCX $n = 10$) and stressed (Ctrl and Sham $n = 10$, OCX $n = 10$) mice. (C) Quantification of blood neutrophils in control (Ctrl and Sham $n = 10$, OCX $n = 10$) and stressed (Ctrl and Sham $n = 10$, OCX $n = 10$) mice. (D) Quantification of bone-marrow GP in control (Ctrl and Sham $n = 10$, OCX $n = 10$) and stressed (Ctrl and Sham $n = 10$, OCX $n = 10$) mice (on the left). Quantification of bone-marrow cMoP in control (Ctrl and Sham $n = 10$, OCX $n = 10$) and stressed (Ctrl and Sham $n = 10$, OCX $n = 10$) mice (on the right). (E) Quantification of blood monocytes in control (Ctrl and Sham $n = 10$, OCX $n = 10$) and stressed (Ctrl and Sham $n = 10$, OCX $n = 10$) mice (Ly6c^{high} on the left, Ly6c^{low} on the right). (F) Quantification of bone-marrow B-cell in control (Ctrl and Sham $n = 10$, OCX $n = 10$) and stressed (Ctrl and Sham $n = 10$, OCX $n = 10$) mice (on the left). Quantification of bone-marrow B-cell precursors in control (Ctrl and Sham $n = 10$, OCX $n = 10$) and stressed (Ctrl and Sham $n = 10$, OCX $n = 10$) mice (on the right). (G) Quantification of bone marrow B-cell precursors in control (Ctrl and Sham $n = 10$, OCX $n = 10$) and stressed (Ctrl and Sham $n = 10$, OCX $n = 10$) mice. (H) Serum IgM titer (on the left) and IgG titer (on the right) in control (Sham=5, OCX $n = 5$) and stressed (Sham = 5, OCX $n = 5$) mice. All data are presented as means \pm SEM. Panels B to G represent two independent experiments. Panel H represents one experiment. Two-way ANOVA multiple comparisons were used to determine statistical significance. ns $p > 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

culminating in increased antibody titers in plasma. Previous work documented that females develop higher antibody responses after immune challenge [7]. However, the potential impact of PS on adaptive immunity and vaccination requires further investigation.

Importantly, a clear consensus on the role of chronic stress on blood immune cells is yet to be obtained. The sex-related changes that we observed could be due to a different level of expression of the glucocorticoid receptor (GR, encoded by NR3C1) and androgen receptor (AR) on progenitors and mature immune cells between female and male mice. Previous studies determined the effect of cell-specific GR deletion in T cells, B cells, monocytes, and neutrophils during acute stress [3], but the sex dimorphism in this response has not yet been determined. AR-deficient mice display neutropenia, further demonstrating the key role of sex hormones in neutrophil generation [16]. Our data revealed that adrenal glands in females produce more corticosterone in comparison to males upon PS. Nevertheless, the underlying mechanisms are not understood. Here, we show for the first time the contribution of androgens on stress response and hematopoiesis, particularly on B-cell and T-cell generation. Nevertheless, ovariectomized females might reveal also the contribution of the estrogens on the PS-induced immune response. How immune cell sex-dimorphism impacts disease development, in particular during autoimmune pathologies characterized by increased prevalence in women, is a critical question that requires further investigation.

Data limitation and future perspectives

An interesting finding of this study is that despite male and female mice being subjected to an identical chronic stress protocol and demonstrating robust immune cell responses, only females exhibited an elevated corticosterone level in both the adrenal gland and plasma 24 h after the final stressor. Nonetheless, the chronic stress response is a dynamic phenomenon wherein both the hypothalamus-pituitary-adrenal axis and the sympathetic nervous system are involved. We believe that males could respond differently to chronic stress exposure or exhibit different dynamic stress-hormone variations over time. Therefore, subsequent research might explore alternative methodologies to evaluate the differential impact of chronic stress on male and female mice, potentially by choosing varied blood sampling time points or by observing changes in behavior.

Experimental model and subject details

Wild-type 7–9-week-old C57BL/6Jrj mice were purchased from Janvier Labs. Janvier Labs performed also the orchidectomies or the mock surgery at 4 weeks of age. When possible, co-housed littermate controls were used. All mice were bred and housed in specific pathogen-free conditions maintained by the animal facility in the Mediterranean Center of Molecular Medicine (INSERM U1065, Université Côte d'Azur). Mice were maintained at ambient temperature of 20–23°C, with 12/12 h light/dark cycles and

food available *ad libitum*. Animal protocols were approved by the French Ministry of Higher Education and Research and by the local ethical committees.

Chronic stress protocol

Mice were exposed to a previously validated protocol of chronic variable stress for 3 weeks [1], followed by a rest period of up to 24 h. Briefly, the following stressors were randomly applied for three consecutive weeks. Isolation: mice were individually housed for 4 h. Damp bedding: water was added to the cage to moisture the bedding without generating large pools. Mice were kept for 6 h with damp bedding. Rapid light–dark changes: using a manual timer, the light was switched at an interval of 7 min for 2 h. Overnight illumination: mice were housed in a separate room with illumination from 7 p.m. to 7 a.m.

Flow cytometry analysis

The different samples were collected 24 h after the last stress. Blood was collected by submandibular bleeding. Red blood cells were lysed using RBC lysing buffer (BD Biosciences). Spleens and thymii were harvested in PBS and were crushed on a 100 μ m sieve. For intracellular cytokine and Foxp3 staining, splenocytes were stimulated with PMA and Ionomycin in the presence of Brefeldin A, then intracellular staining was performed using the Foxp3 staining kit. The bone marrow was flushed from one tibia and one femur using FACS buffer (PBS containing 0.3 mM EDTA and 0.06% BSA). Then, bone marrow cells were filtered using a 100 μ m sieve. The resulting suspensions were centrifuged (400 \times g, 5 min, 4°C) and RBCs were lysed using RBC lysis buffer. Cells were then resuspended in FACS buffer and stained for 30 min at 4°C in the dark and then washed. A viability stain (DAPI or Zombie viability dye) was used whenever possible. Conventional flow cytometry data were acquired on a BD FACS Canto II and spectral flow cytometry data were acquired on a Cytex Aurora cytometer. We adhered to the “Guidelines for the use of flow cytometry and cell sorting in immunological studies” (<https://onlinelibrary.wiley.com/doi/full/10.1002/eji.202170126>; [17]). All analyses were performed using FlowJo software (Tree Star).

ELISA assays

Blood was collected by submandibular bleeding. One adrenal gland was crushed in 200 μ L PBS using 0.1 mm glass beads and a precellys homogenizer (Biospec cat# 11079101). Corticosterone, norepinephrine, and IL-6 levels were measured in plasma and adrenal gland homogenates as instructed (R&D Systems Cat# KGE009, Abnova Cat# CKA3836, and R&D Systems Cat# DY406, respectively).

For Ig, standard sandwich ELISA was performed using coated anti-mouse IgG (Jackson ImmunoResearch #115006062) and anti-mouse IgM (Jackson ImmunoResearch #115006075). Serial

Key resources table

Reagent or resource	Source	Identifier
Antibodies:		
CD45 APC-Fire810 (Clone 30-F11)	Biologend	Cat# 103174
CD11b BV750 (Clone M1/70)	Biologend	Cat# 101263
CD11b PeCy5 (Clone M1/70)	Biologend	Cat# 101210
Ly6G BV785 (Clone 1A8)	Biologend	Cat# 127645
CD115 PE (Clone AFS98)	Biologend	Cat# 135506
CD115 BV711 (Clone AFS98)	Biologend	Cat# 135515
Ly6C BV570 (Clone HK1.4)	Biologend	Cat# 128030
Ly6C BV711 (Clone HK1.4)	Biologend	Cat# 128037
Ly6C BV605 (Clone HK1.4)	Biologend	Cat# 128035
CD4 AF700 (Clone RM4-5)	Biologend	Cat# 100536
CD8 AF647 (Clone 53-6.7)	Biologend	Cat# 100724
CD8 BV510 (Clone 53-6.7)	Biologend	Cat# 100752
CD8 FITC (Clone YTSI56.7.7)	Biologend	Cat# 126605
TCRb PB (Clone H57-597)	Biologend	Cat# 109226
TCRb FITC (Clone H57-597)	Biologend	Cat# 109206
TCRb PeCy5 (Clone H57-597)	eBioscience	Cat# 15-5961-81
CD19 BVV737 (Clone 1D3)	Biologend	Cat# 612782
B220 PeCy5 (Clone RA3-6B2)	eBioscience	Cat# 15-0452-82
B220 FITC (Clone RA3-6B2)	eBioscience	Cat# 11-0452-85
IgM PE (Clone RMM-1)	Biologend	Cat# 406507
CD24 BVV496 (Clone M1/69)	BD Biosciences	Cat# 612953
CD43 AF700 (Clone Ly48)	Biologend	Cat# 143213
CD16/32 PercpCy5.5 (Clone 93)	Biologend	Cat# 101324
CD34 BV421 (Clone SA376A4)	Biologend	Cat# 152208
cKit APC/Cy7 (Clone 2B8)	Biologend	Cat# 105825
Sca1 BV650 (Clone D7)	Biologend	Cat# 108143
CD150 PeCy7 (Clone TC15-12F12.2)	BD Biosciences	Cat# 560518
CD48 AF488 (Clone HM48-1)	Biologend	Cat# 103414
CD135 PerCP-eFluor710 (Clone A2F10)	Invitrogen	Cat# 46-1351-82
CD11c PeCy7 (Clone N418)	Biologend	Cat# 117318
F4/80 BV650 (Clone BM8)	Biologend	Cat# 123149
MerTK PE (Clone REA477)	Miltenyi	Cat# 140-123-777
CD64 BV711 (Clone X54-5/7.1)	Biologend	Cat# 139311
MHC-II VioBlue (Clone REA813)	Miltenyi	Cat# 130-112-394
TCRgd BV605 (Clone GL3)	Biologend	Cat# 118129
CD44 SuperBright645 (Clone IM7)	eBioscience	Cat# 64-0441-82
CD25 BV510 (Clone PC61)	Biologend	Cat# 102041
Foxp3 AF647	eBioscience	Cat#51-5773-82
IL-17 PE	BD Biosciences	Cat# 559502
IFNg APC	eBioscience	Cat# 17-7311-82
IL-4 BV421	Biologend	Cat# 504120
AffiniPure F(ab') ₂ fragment Goat anti-Mouse IgG (H+L)	Jackson Immunoresearch	Cat#115-006-062
AffiniPure F(ab') ₂ fragment Goat anti-Mouse IgM, μ chain specific	Jackson Immunoresearch	Cat#115-006-075
Alkaline phosphatase AffiniPure Goat anti-Mouse IgG, light chain specific	Jackson Immunoresearch	Cat#115-055-174
Chemicals, peptides, and recombinant proteins:		
DAPI	Sigma	Cat# D9542
Zombie Violet Fixable Viability Kit	Biologend	Cat# 423114
Bovine Serum Albumin (BSA)	Sigma	Cat# A7030
Lysis Buffer	BD Biosciences	Cat# 555899
PNPP substrate	Sigma	Cat#N2770
Critical commercial assays		
Norepinephrine ELISA Kit	Abnova	Cat# KA3836
Corticosterone Parameter Assay Kit	R&D Systems	Cat# KGE009
Mouse IL-6 DuoSet ELISA	R&D Systems	Cat# DY406
Foxp3 Staining Buffer Set	Miltenyi	Cat# 130-093-142
Software and algorithms		
Prism 10	GraphPad	N/A
FlowJo	Tree Star	N/A
SpectroFlo	Cytek	N/A

sera dilutions were tested and revealed using AP-coupled anti-mouse Ig Light chain (Jackson ImmunoResearch # 115-055-174) and PNPP substrate (SIGMA #N2770). Titers were extracted in GraphPad using nonlinear fit IC50 measure and plotted as LOG titers.

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Author contributions: Bastien Dolfi and Stoyan Ivanov designed the study. BD and SI wrote the manuscript and prepared the figures. Bastien Dolfi, Alexandre Gallerand, Zakariya Caillot, Alexia Castiglione, Fairouz N. Zair, Lorlana Leporati, Margaux Giacchero, Eloïse Goës, Axelle Strazulla, Rodolphe R. Guinamard, Adeline Bertola, and Stoyan Ivanov performed experiments. Bastien Dolfi, Alexandre Gallerand, David Dombrowicz, Rodolphe R. Guinamard, Adeline Bertola, and Stoyan Ivanov analyzed the data and edited the manuscript. Stoyan Ivanov obtained funding for the project.

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Abbreviations: **BM:** bone marrow · **GP:** granulocyte progenitors · **GR:** glucocorticoid receptor · **HSC:** hematopoietic stem cells · **PS:** psychosocial stress

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