Sympathetic Release of Splenic Monocytes Promotes Recurring Anxiety Following Repeated Social Defeat

Daniel B. McKim, Jenna M. Patterson, Eric S. Wohleb, Brant L. Jarrett, Brenda F. Reader, Jonathan P. Godbout, and John F. Sheridan

ABSTRACT

BACKGROUND: Neuroinflammatory signaling may contribute to the pathophysiology of chronic anxiety disorders. Previous work showed that repeated social defeat (RSD) in mice promoted stress-sensitization that was characterized by the recurrence of anxiety following subthreshold stress 24 days after RSD. Furthermore, splenectomy following RSD prevented the recurrence of anxiety in stress-sensitized mice. We hypothesize that the spleen of RSD-exposed mice became a reservoir of primed monocytes that were released following neuroendocrine activation by subthreshold stress.

METHODS: Mice were subjected to subthreshold stress (i.e., single cycle of social defeat) 24 days after RSD, and immune and behavioral measures were taken.

RESULTS: Subthreshold stress 24 days after RSD re-established anxiety-like behavior that was associated with egress of Ly6C<sup>hi</sup> monocytes from the spleen. Moreover, splenectomy before RSD blocked monocyte trafficking to the brain and prevented anxiety-like behavior following subthreshold stress. Splenectomy, however, had no effect on monocyte accumulation or anxiety when determined 14 hours after RSD. In addition, splenocytes cultured 24 days after RSD exhibited a primed inflammatory phenotype. Peripheral sympathetic inhibition before subthreshold stress blocked monocyte trafficking from the spleen to the brain and prevented the re-establishment of anxiety in RSD-sensitized mice. Last, β-adrenergic antagonism also prevented splenic monocyte egress after acute stress.

CONCLUSIONS: The spleen served as a unique reservoir of primed monocytes that were readily released following sympathetic activation by subthreshold stress that promoted the re-establishment of anxiety. Collectively, the long-term storage of primed monocytes in the spleen may have a profound influence on recurring anxiety disorders.

Keywords: Anxiety, Macrophages, Microglia, Neuroinflammation, PTSD, Stress

http://dx.doi.org/10.1016/j.biopsych.2015.07.010
populations (13). Similarly, RSD promotes a primed monocyte phenotype characterized by exaggerated inflammatory response to ex vivo innate immune challenge that is resistant to inhibition by GCs (27). Additionally, the development of prolonged anxiety-like behavior that is detectable up to 8 days after RSD (28) is dependent upon sympathetic activation of the immune system (13,25,27). Further studies revealed that the development of prolonged anxiety-like behavior was specifically dependent on monocyte accumulation in the brain following RSD (29). Taken together, monocyte trafficking to the brain represents a novel axis of immune-to-brain signaling that promotes prolonged behavioral responses to stress (30,31).

Recent evidence shows that RSD caused long-term sensitization that caused mice to have exaggerated immunological and behavioral responses following subsequent exposure to an acute stressor (28). In this study, RSD-exposed mice were termed stress-sensitized because they exhibited exaggerated responses to an otherwise subthreshold stressor. For instance, exposure to a single cycle of social defeat 24 days after RSD re-established monocyte trafficking and anxiety-like behavior without affecting these parameters in naïve, non-stressed control mice (28). Notably, splenectomy in stress-sensitized mice prevented the re-establishment of monocyte trafficking and anxiety-like behavior 24 days after RSD. These data were interpreted to indicate that monocyte trafficking from the spleen to the brain promoted the re-establishment of anxiety in stress-sensitized mice. However, it is currently unclear if the spleen is unique in its ability to store these releasable monocytes. In immunological studies, other immune organs were capable of storing myeloid cells, but the spleen was unique in its capacity to functionally contribute monocytes to distant inflammatory sites (32-35).

Based on these collective data, the objective of this study was to test the hypothesis that the spleen of RSD-exposed mice serves as a unique reservoir of primed monocytes that are released following sympathetic outflow in response to an acute stressor. Here, we provide several lines of evidence that the spleen is unique in its capacity to maintain and release a population of primed monocytes 24 days after RSD. Moreover, subthreshold stress in mice caused this pool of primed monocytes to traffic to the brain and promote the recurrence of anxiety-like behavior. Furthermore, inhibition of the peripheral sympathetic nervous system during subthreshold stress blocked spleen-to-brain monocyte trafficking and prevented the recurrence of anxiety in stress-sensitized mice. These novel studies reveal that the spleen is capable of maintaining long-term neuroimmune sensitization that can regulate behavioral responses many days after the initial sensitizing event.

METHODS AND MATERIALS

Mice

Male C57BL/6 (6–8 weeks old) and CD-1 (retired breeders) mice were purchased from Charles River Laboratories (Wilmington, Massachusetts). C57BL/6 mice were housed in cohorts of three per cage. All procedures were in accordance with the National Institutes of Health Guidelines and were approved by the Ohio State University Institutional Laboratory Animal Care and Use Committee.

Repeated Social Defeat

Mice were subjected to RSD as previously reported (29) and as described in Supplement 1. In brief, an aggressive intruder male CD-1 mouse was introduced into cages of established male cohorts (three per cage) of C57BL/6 mice for 6 consecutive nights. During each cycle, submissive behaviors were observed to ensure that the resident mice showed subordinate behavior. As previously described (28), to study the sensitizing effects of RSD, mice were either exposed to control (naïve) or RSD conditions (stress-sensitized [SS]). Then, 24 days later naïve and SS mice were subjected to an additional cycle of social defeat. All behavior and biological measures were obtained 14 hours after the final cycle. This time point was selected because both hypothalamus-pituitary-adrenal axis and sympathetic nervous system (SNS) activation following social defeat return to baseline within 14 hours (27).

Guanethidine Treatment

Twenty-four hours before acute social defeat, mice were injected subcutaneously with either vehicle or 50 mg/kg guanethidine (Santa Cruz Biotechnology, Dallas, Texas). Injection regimen was based on a previous report (36).

Anxiety-like Behavior

Anxiety-like behavior was determined using open-field activity as previously reported (29) and as described in Supplement 1.

Isolation of Cells from Bone Marrow, Spleen, Blood, and Brain

Tissues were collected immediately following carbon dioxide asphyxiation. Cells from bone marrow (BM), spleen, and blood were isolated as previously described (26,27). CD11b<sup>+</sup> brain cells were enriched by Percoll density gradient as previously reported (29). See Supplement 1 for details.

Statistical Analysis

To determine significant main effects and interactions between main factors, data were analyzed using two-way analysis of variance using the general linear model procedures of SAS (SAS Institute Inc., Cary, NC). Analysis of variance results are presented in figure legends. When there was a main effect of experimental treatment or a treatment interaction effect, differences between means were evaluated by an F-protected t test using the least-significant difference procedure of SAS. All data are expressed as treatment means ± SEM.

RESULTS

Recurrence of Anxiety-like Behavior in Stress-Sensitized Mice Was Associated with Ly6<sup>C</sup> Monocyte Egress from the Spleen

Our previous study showed that removal of the spleen after RSD prevented both monocyte trafficking to the brain and the recurrence of anxiety-like behavior in stress-sensitized
mice (28). To further examine the possible release of monocytes from the spleen in response to acute stress, the following experimental design was used. Figure 1A illustrates that mice were stress-sensitized by six cycles of social defeat (SS) or left undisturbed (naïve). Mice were subjected to acute social defeat 24 days later and anxiety-like behavior and biochemical analyses were completed 14 hours later. Stress-sensitized mice exposed to acute social defeat exhibited anxiety-like behavior in the open field with increased time to enter the center (interaction, $F_{1,42} = 4.52, p < .05$) and reduced time spent in the center (tendency for interaction, $F_{1,44} = 2.98, p < .10$). (D) Acute social defeat in SS mice increased percentage of macrophages associated with the brain (interaction, $F_{1,21} = 8.22, p < .05$) and (E) increased Ly6C$^+$ monocytes in circulation (main effect of SS, $F_{1,19} = 4.47, p < .05$; tendency for interaction, $F_{1,19} = 2.67, p < .1$). Several inflammatory mediators were determined in a coronal brain section and acute social defeat increased messenger RNA (mRNA) expression of IL-1β in SS mice ($F_{1,36} = 10.55, p < .01$; interaction, $F_{1,36} = 3.66, p < .05$), CCL2 ($F_{1,36} = 5.42, p < .05$), TNF-α (interaction, $F_{1,39} = 4.23, p < .05$), and CD14 (interaction, $F_{1,39} = 4.46, p < .05$). The relative number of Ly6C$^+$ monocytes was determined in the (G) spleen and (H) bone marrow (BM). Acute stress reduced the number of monocytes in both the spleen (interaction, $F_{1,18} = 8.35, p < .01$) and bone marrow (interaction, $F_{1,18} = 9.92, p < .01$) of SS mice. (I) Spleen weight was determined and shown as a percentage of body mass. Bars represent the mean ± SEM. Means with asterisk (*) are significantly different from control mice ($p < .05$) and means with (#) tended to be different from control mice ($p < .1$), according to F-protected post hoc analysis.
Splenectomy Before RSD Prevented Recurrence of Monocyte Trafficking and Anxiety-like Behavior Following Acute Stress in Sensitized Mice

Our previous study showed that removal of the spleen after RSD prevents monocyte accumulation in the brain following acute stress 24 days later (28). It is possible, however, that other immune compartments may compensate for the spleen following splenectomy and function as alternative myeloid reservoirs (35). Thus, the next objective was to determine if other immune reservoirs compensated for the spleen during stress. To do this, mice were splenectomized 14 days before RSD and then exposed to acute social defeat 24 days later (Figure 2A). Splenectomy before RSD prevented the recurrence of monocyte trafficking and anxiety-like behavior in SS mice. For instance, acute stress in sham-treated SS mice increased Ly6C<sup>hi</sup> monocytes in circulation ($p < .05$; Figure 2B,C), increased brain macrophages ($p < .05$; Figure 2D,E), and increased IL-1β, TNF-α, and CD14 mRNA expression in the brain (all $p < .1$; Figure 2F), all of which were prevented by splenectomy (Figure 2B–F). Moreover, prevention of monocyte trafficking to the brain corresponded with prevention of the recurrence of anxiety-like behavior. For instance, sham SS mice tended to take longer to enter the center ($p = .1$; Figure 2H) compared with naive sham mice. Moreover, sham SS mice also had reduced time spent in the center of the open field compared with all other groups ($p < .05$; Figure 2I). This anxiety-like behavior, however, was undetected in splenectomized SS mice (Figure 2H,I). These data are interpreted to indicate that other immune compartments were unable to compensate for the spleen and act as functional reservoirs of releasable monocytes following RSD.

Splenectomy Did Not Influence Monocyte Trafficking or Anxiety-like Behavior 14 Hours After RSD

Our data indicate that monocyte release and anxiety-like behavior following acute stress in SS mice were dependent on the spleen (Figure 2). It is possible that the spleen was also necessary for some of the primary immune and behavioral responses to the initial exposure to RSD. To address this, mice were splenectomized before RSD, and behavioral and biological measures were determined 14 hours after the final cycle (Figure 3A). Consistent with substantial accumulation of primed myeloid cells in the spleen (26), RSD increased spleen weight in sham mice ($p < .05$; Figure 3B). Overall, Figure 3 shows that the primary immune and behavioral responses evident 14 hours after RSD were unaltered by splenectomy. For example, there was a main effect of RSD on myelopoiesis with increased monocytes and granulocytes and decreased lymphocytes and erythrocytes in the bone marrow (all $p < .05$; Figure 3C,D) that was unaffected by the splenectomy. In addition, splenectomy did not prevent increased Ly6C<sup>hi</sup> monocytes in circulation following RSD (Figure 3E), did not affect the accumulation of macrophages in the brain (Figure 3F,G), and did not prevent increased brain cytokine mRNA expression of IL-1β, interleukin-6 (IL-6), TNF-α, CD14, and CCL2 (data not shown). Nor did splenectomy block the development of anxiety-like behavior 14 hours after RSD (Figure 3H,I). Taken together, the spleen was not required for the primary immune and behavioral responses to RSD observed 14 hours after the last cycle.

RSD Increased the Accumulation of Primed Myeloid Cells in the Spleen that Exhibited Exaggerated Inflammatory Response to Ex Vivo Mitogen Challenge

Data presented here demonstrate that the spleen is necessary for the maintenance of a releasable pool of monocytes following RSD. Previous reports indicated that RSD increased release and trafficking of BM-derived monocyte-lineage cells that were both primed and GC-insensitive (26,37,38). For instance, our previous results showed that primed monocytes seed the spleen and retain a GC-insensitive phenotype for at least 8 days after RSD (39). Nonetheless, the presence and phenotype of monocytes in the spleen 24 days after RSD is unknown. To address this, cytokine responses to lipopolysaccharide (LPS) and sensitivity to GCs were assessed in spleen and bone marrow cells 24 days after RSD (Figure 4A). IL-6 production following ex vivo LPS stimulation of BM was not different between groups (Figure 4B). However, splenocytes from SS mice produced more IL-6 following LPS stimulation compared with cells from naïve mice ($p < .05$; Figure 4C). Additionally, there was increased cell viability in response to LPS stimulation in splenocytes from SS mice compared with those from naïve mice ($p < .05$; Figure 4D). This exaggerated splenocyte response to LPS stimulation was associated with enhanced baseline mRNA expression of CD14 ($p < .05$; Figure 4E) but not toll-like receptor 4 (data not shown). Next, to determine if this primed phenotype was associated with GC insensitivity in SS mice, the effect of increasing corticosterone concentrations on LPS-induced IL-6 production and cell viability was determined. Figures 4F and 4G show that increasing corticosterone concentrations reduced cell viability ($p < .05$; Figure 4F) and IL-6 production ($p < .05$; Figure 4G) independent of stress-sensitization. Thus, primed but not GC-insensitive monocytes were maintained in the spleen for at least 24 days after RSD.

Sympathetic Inhibition Prevented Monocyte Trafficking and the Recurrence of Anxiety-like Behavior in SS Mice

Data shown here indicate that the spleen is uniquely responsible for the increased availability of primed and releasable monocytes 24 days after RSD. Despite this, the physiological signaling pathway that initiates release of monocytes from the spleen in response to acute stress is unknown. Previous studies demonstrated a role for the SNS in the release of splenic myeloid cells (40). Therefore, guanethidine, a peripheral sympathetic inhibitor, was used because it is a non-central nervous system active drug that prevents the release of norepinephrine (NE) by vesicular displacement (41). In this manner, guanethidine inhibits SNS activation in a dose-dependent manner under both homeostasis and stress.

Therefore, the effect of acute stress in RSD-sensitized mice was determined following guanethidine intervention (Figure 5A). Figure 5B confirms that guanethidine intervention significantly reduced splenic NE at 12 and 24 hours after injection in mice exposed to acute stress ($p < .05$; Figure 5J).
As expected, acute stress increased Ly6C<sup>hi</sup> monocytes in circulation (p < .05; Figure 5C) and increased CD45<sup>hi</sup> brain macrophages in SS mice but not naïve mice (p < .05; Figure 5D,E). This redistribution of monocytes, however, was undetected in guanethidine-treated SS mice (Figure 5C–E).

Similarly, acute stress increased brain mRNA expression of IL-1β and TNF-α in vehicle-treated SS mice (both p < .05; Figure 3F). This was also prevented by guanethidine treatment. Moreover, blockade of monocyte trafficking to the brain with guanethidine corresponded with prevention of anxiety-like behavior in SS mice. For instance, acute stress in vehicle-treated SS mice increased time to enter the center (p < .05; Figure 5G) and reduced time spent in the center of the open field (p < .05; Figure 5H), and neither of these behaviors were...
observed in guanethidine-treated SS mice (Figure 5G,H). To further address the role of the SNS in the release of monocytes from the spleen, the effect of pretreatment with propranolol, a beta adrenergic receptor antagonist, was determined. Propranolol pretreatment (1 hour) before acute stress in SS mice enhanced monocyte retention in the spleen (\(p < .05\); Figure S1A in Supplement 1) and reduced the presence of Ly6Chi monocytes in circulation (Figure S1B in Supplement 1). Taken together, sympathetic inhibition prevented spleen-to-brain monocyte trafficking, and this corresponded with attenuated anxiety-like behavior and reduced neuroinflammatory signaling following acute stress exposure.

**DISCUSSION**

The results presented here demonstrate a novel and critical role for the spleen in the maintenance of stress sensitization that persisted for 24 days after the initial sensitizing, stressful event. First, the recurrence of anxiety-like behavior was associated with increased monocyte trafficking from the...
spleen and increased macrophage accumulation in the brain. Next, novel data shown here indicate that the spleen was indispensable for the maintenance of primed and releasable monocytes 24 days after RSD. For example, splenectomy before stress sensitization blocked monocyte redistribution and prevented the recurrence of anxiety in stress-sensitized mice. Notably, no other organ acted as a compensatory reservoir. Additionally, splenectomy before RSD did not attenuate the primary immune and behavioral response to RSD observed 14 hours after the final cycle. Thus, the spleen was necessary for the maintenance of releasable monocytes 24 days after RSD but was not necessary for the initial production and trafficking of primed monocytes or the initial development of anxiety immediately after RSD. In addition, the splenic monocytes retained a primed but not GC-insensitive phenotype in stress-sensitized mice. This was interpreted to indicate that RSD primed and mobilized monocyte-lineage cells that persisted in the spleen for 24 days following cessation of the stressor. Further work addressed physiological signals that contributed to the release of monocytes from the spleen. These studies showed that pretreatment with the SNS inhibitor, guanethidine, prevented monocyte trafficking and anxiety in stress-sensitized mice. Thus, we interpret these data to mean that sympathetic initiation of monocyte trafficking from the spleen to the brain promoted the recurrence of anxiety-like behavior in sensitized mice.

An important finding in this study was that stress sensitization following RSD was associated with an altered myeloid composition of the spleen. First, there was a tendency for increased Ly6C\textsuperscript{hi} monocytes in the spleen that persisted 24 days after exposure to RSD. Second, accumulation of monocytes in circulation and the brain following acute stress in stress-sensitized mice was associated with a robust reduction in the number of Ly6C\textsuperscript{hi} monocytes in the spleen. This is consistent with egress of Ly6C\textsuperscript{hi} monocytes from the spleen that accumulated in circulation and the brain. This redistribution of splenic monocytes characterized here resembles studies of myocardial infarction that revealed that monocyte redistribution from the spleen contributed to myocardial pathogenesis (32). Notably, acute stress in stress-sensitized mice also reduced the number of Ly6C\textsuperscript{hi} monocytes in the BM. Nonetheless, our previous work (28) and data presented here show that cells from the spleen but not the BM are critical for increased trafficking of primed monocytes in stress-sensitized mice. Importantly, these splenic monocytes released by acute stress contribute to the recurrence of anxiety in the open field (latency and time spent in the center).

The splenectomy studies presented here provide evidence that the spleen is not required for primary immune and
behavioral responses to RSD, but rather, the spleen is necessary for the maintenance of releasable monocytes 24 days after RSD. This is an important distinction, because it implicates the BM, not the spleen, in the initial production and accumulation of monocytes immediately following RSD. These results are consistent with other studies of RSD and chronic unpredictable stress that demonstrated increased production of myeloid cells in the BM (16,26). In addition, RSD suppressed T cell production in the BM independent of splenectomy. Others have reported that T cells are capable of regulating behavior following social defeat stress (42). This further supports the hypothesis that immune-derived signals

Figure 5. Guanethidine blocked primed monocyte trafficking from the spleen to the brain and prevented the re-establishment of anxiety in stress-sensitized (SS) mice. (A) Male C57BL/6 mice were stress-sensitized by six repeated cycles of social defeat or left undisturbed as control subjects (naive). Mice were pretreated with guanethidine (Guan) or vehicle (Veh) before acute social defeat. Fourteen hours after acute social defeat, anxiety-like behavior and biochemical analyses were completed. (B) Splenic norepinephrine was determined immediately following acute stress at 12, 24, and 48 hours following guanethidine injections (50 mg/kg). (C) The percentage of Ly6C<sup>hi</sup> monocytes was determined in blood. Acute stress increased percent Ly6C<sup>hi</sup> monocytes in SS-vehicle mice but not naive mice (interaction effect, F<sub>1,46</sub> = 4.35, p < .05). (D) Representative flow bivariate dot plots of CD11b and CD45 labeling on enriched brain macrophages (MΦ) and microglia (MGL). (E) The percentage of brain macrophages was determined and they were increased by acute stress (F<sub>1,46</sub> = 14.66, p < .0005) and this effect was blocked by guanethidine (F<sub>1,46</sub> = 7.72, p < .01). (F) Several inflammatory mediators were determined in a coronal brain section and acute social defeat increased messenger RN (mRNA) expression of IL-1β (F<sub>1,22</sub> = 6.46, p < .05) and TNF-α (F<sub>1,22</sub> = 3.18, p < .1) in SS-vehicle mice but not SS-Guan mice. (G, H) SS vehicle-treated mice exhibited anxiety-like behavior in the open field with increased time to enter the center (G) (F<sub>1,46</sub> = 2.42, p ≤ .1; main effect of splenectomy; F<sub>1,46</sub> = 5.66, p < .05) and reduced time spent in the center (H) (tendency for interaction effect, F<sub>1,46</sub> = 2.42, p ≤ .1). Bars represent the mean ± SEM. Means with asterisk (*) are significantly different from control mice (p < .05) and means with (#) tended to be different from control mice (p < .1), according to F-protected post hoc analysis. RSD, repeated social defeat.
are important regulators of stress-related behaviors. Data from RSD indicated that the monocytes that accumulate with stress are primed to be more inflammatory in response to challenges (e.g., LPS) and less sensitive to the anti-inflammatory effects of GCs (27). Thus, we hypothesize that RSD mobilizes primed monocytes that seed the spleen and contribute to the maintenance of releasable monocytes with the ability to traffic in the brain and promote anxiety in stress-sensitized mice.

Related to the above points, data here support the hypothesis that splenic monocytes from stress-sensitized mice are inherently more reactive to neuroendocrine or immune stimulation. For instance, cells that persist in the spleen 24 days after RSD appear to have a primed profile with increased IL-6 secretion following ex vivo LPS stimulation. In contrast, BM cells from stress-sensitized mice were not more sensitive to LPS stimulation. It is important to mention that these experiments were completed with whole splenocytes, but we attribute these effects to monocytes. This is supported by previous studies showing that monocytes/macrophages were the primary cells that responded to LPS stimulation in ex vivo splenocyte cultures (43). Although stress-sensitized mice retained a primed monocyte phenotype, they did not retain the GC-insensitive phenotype that is observed for up to 8 days after RSD (27,39). We interpret these data to indicate that the spleen maintains a population of primed monocytes following stress sensitization and that these cells can traffic to the brain and promote the recurrence of anxiety following acute stress many days later. Despite the evidence provided here, it is possible that enhanced splenic monocyte trafficking observed in stress-sensitized mice is mediated by neuroendocrine sensitization and is unrelated to immunomodulation. For example, fear conditioning in stress-sensitized mice might contribute to exaggerated neuroendocrine response to the acute stressor, resulting in sufficient stimulation to cause the release of splenic monocytes that traffic to the brain and promote anxiety. Nonetheless, priming of splenic monocytes was observed independent of neuronal mediation. For instance, splenic myeloid cells demonstrated increased CD14 mRNA expression and enhanced IL-6 production following ex vivo LPS stimulation. Thus, persistent splenic priming was observed independent of neuroendocrine sensitization.

Another important finding was that the release of primed monocytes from the spleen of stress-sensitized mice after acute social defeat was dependent activation of the β-adrenergic receptors of the SNS. Our previous work with RSD shows that monocyte redistribution is dependent on SNS activation (25,27,44). In addition, activation of the SNS specifically has been implicated in splenic monocyte egress (40). Moreover, SNS inhibition, but not adrenalectomy, prevented myeloid redistribution following social defeat in rats (45). While there is evidence that GCs are important for transient leukocyte redistribution following acute stress (46), data here show a primary role for the SNS in the egress of primed splenic monocytes in sensitized mice. The SNS can interact with the spleen either through circulating epinephrine or norepinephrine released from the adrenal medulla or through direct sympathetic innervation (47). Here, we confirmed that guanethidine intervention before acute stress depleted splenic NE 12 and 24 hours postinjection. These results mirror previous reports that subcutaneous guanethidine substantially reduced splenic NE for more than 24 hours (48). Our guanethidine intervention studies showed that monocyte release from the spleen was dependent on SNS activation. In addition, pretreatment with propranolol, a β-adrenergic receptor antagonist, before acute stress in SS mice enhanced monocyte retention in the spleen and reduced the presence of Ly6C$^hi$ monocytes in circulation. It should be noted that the relative contribution of circulating NE versus direct splenic innervation cannot be discerned with data presented here. For instance, guanethidine prevents NE release in both tissue and circulation (41), and others have implicated circulating NE in splenic monocyte egress (40). Taken together, monocyte egress from the spleen with acute stress was dependent upon SNS activation of β-adrenergic receptors.

Data here show that guanethidine blocked accumulation of Ly6C$^hi$ monocytes in circulation and blocked macrophage trafficking in the brain. In addition, this blockade corresponded with prevention of anxiety-like behavior in stress-sensitized mice. This point is of particular interest because it reveals a clinically relevant pharmacologic strategy to attenuate maladaptive behaviors related to peripheral immunological sensitization. Although underappreciated, it has been reported that β-adrenergic antagonists (i.e., beta-blockers) have chronic anxiolytic effects in certain clinical populations (49) that may be related to interactions with the immune system. Thus, studies here provide a biological mechanism that supports the use of sympathetic inhibitors to abrogate recurring anxiety promoted by monocyte redistribution. Thus, we conclude that activation of the SNS is the key parameter for the release of primed monocytes from the spleen of stress-sensitized mice.

Overall, the current studies provide evidence that the spleen contributes to long-term neuroimmune sensitization capable of regulating behavioral responses many days after a sensitizing stressful event. For example, the spleen acted as a unique reservoir for maintaining primed monocytes following exposure to RSD. These primed monocytes were readily releasable following neuroendocrine activation by acute stress 24 days after RSD. Neuroendocrine activation by acute stress caused primed monocytes to traffic to the brain and promote the recurrence of anxiety in sensitized mice. This phenomenon may be relevant because persistent or recurring behavioral complications observed in several psychiatric populations are associated with immune activation (50). Thus, recurring behavioral complications associated with psychological stress may be related to splenic monocyte redistribution. Collectively, these findings reveal novel neuroimmune mechanisms that may be implicated in recurring anxiety disorders.

ACKNOWLEDGMENTS AND DISCLOSURES

This study was supported by National Institute of Mental Health Grants R01-MH093473 and R01-MH097243 to JFS. DBM and BLJ were supported by a National Institute of Dental and Craniofacial Research Training Grant T32-DE014320.

We thank Dr. Kelley Madden and Ryan Dawes (University of Rochester) for their technical assistance measuring norepinephrine.

All authors report no biomedical financial interests or potential conflicts of interest.
ARTICLE INFORMATION

From the Division of Biosciences (DBM, JMP, ESW, BLJ, BFR, JFS), College of Dentistry, and Department of Neuroscience (DBM, JMP, ESW, BLJ, JFS), College of Medicine, The Ohio State University, Columbus, Ohio; Department of Psychiatry (ESW), School of Medicine, Yale University, New Haven, Connecticut; and Institute for Behavioral Medicine Research (JPG, JFS), and Center for Brain and Spinal Cord Repair (JPG, JFS), The Ohio State University, Columbus, Ohio.

Address correspondence to John F. Sheridan, Ph.D., The Ohio State University, Division of Oral Biology, College of Dentistry, 223 IBMR Building, 460 Medical Center Drive, Columbus, OH 43210; E-mail: sheridan.18@osu.edu; John.Sheridan@osumc.edu.

Received Feb 26, 2015; revised Jun 19, 2015; accepted Jul 7, 2015.

Supplementary material cited in this article is available online at http://dx.doi.org/10.1016/j.biopsych.2015.07.010.

REFERENCES


