Substantial evidence indicates that stress can precipitate or worsen symptoms of inflammation in general and more specifically in multiple sclerosis (MS), a demyelinating, autoimmune disease characterized by inflammation of the central nervous system (CNS). However, the mechanism of how stress affects MS is not well understood. We reviewed publications in PubMed since 1995 and propose that neuropeptides secreted under stress, such as corticotropin releasing hormone (CRH) and neurotensin (NT), activate microglia and mast cells to release inflammatory molecules. These lead to maturation and activation of T17 autoimmune cells, disruption of the blood–brain barrier (BBB) and T cell entry into the CNS, thus promoting brain inflammation and contributing to MS pathology. Reduction of stress and inhibition of these processes by select flavonoids could provide novel therapeutic approaches.

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such as infections, toxins, immunizations, trauma, sunlight exposure and hormonal variables have been implicated [2]. Although MS affects young women more often than men, male gender has been associated with a poorer prognosis. This could be attributed to a possible protective role that estrogen or progesterone may have in the severity of the disease, but not in the risk of MS [3–5].

Increasing evidence indicates that stress can worsen immunity [6] and brain inflammation [7,8], which is important in the pathogenesis of MS [9], and neuropsychiatric disorders in general [10,11]. We reviewed publications in PubMed since 1995 that report any association between stress, neuropeptides, microglia, mast cells and MS.

2. Correlation between stress and MS

2.1. Human studies

Increasing evidence indicates that symptoms in relapsing–remitting MS may be precipitated or exacerbated by stress [12]. A meta-analysis of 14 studies (case–control and longitudinal prospective) published in 2004, showed that there is a significant association between stressful life events and subsequent relapses of MS in humans [13]. A cohort study in Denmark examined the association between MS and a major stressful life event, the death of a child. The study comprised 21,062 parents who had lost a child and 293,745 matched parents who had not lost a child. The study indicated a significantly higher MS risk in parents who lost a child than in parents who did not for at least 8 years [14]. MS relapses, in a population of 50 female MS patients in the USA, were more likely during at-risk periods following stressful life events and were relatively independent of the threat level and type of stressor [15]. In a study conducted in Greece, cumulative stressful life events were shown to pose a greater risk for relapse in ambulatory women with relapsing–remitting MS [16]. Duration was the only stress attribute that seemed to increase the risk for relapsing in contrast to stress type and stress severity [16]. In another Greek study, 37 female patients kept diaries of stressful life events and anxiety levels that were subsequently ranked according to the Holmes and Rahe Social Readjustment Rating Scale and the Hamilton Rating Scale for Anxiety, respectively. Multiple reported stressful life events and elevated levels of anxiety were each found to be significantly associated with increased risk for relapse of MS [17]. A Lebanese study of 216 patients showed an increase in both clinical relapses and MRI disease activity in patients with MS during periods of war stress [18]. This is in line with an earlier study of 156 patients in Israel that reported that civilian exposure to war stress is associated with increased risk for MS relapses [19].

In a case–control study of 100 MS patients compared to hospital controls, significantly more MS patients reported that they were under unusual stress in the 2 year period prior to onset age [20]. Also, a comparison of 95 pairs of MS patients revealed that patients in relapse scored higher on emotional disturbance and intensity of stressful events than patients in remission [21]. An American study of 55 MS patients showed that patients who experienced qualitatively extreme stressful events were 37 times more likely to relapse than those not exposed to such events [22]. A comparison of 39 patients with early MS and 40 matched non-patient volunteers in another American study revealed that the proportion of MS patients who experienced marked life adversity in the year prior to onset of symptoms was significantly higher than for non-patients in the year before interview [23]. Similar results were reported in a study of 73 patients in Netherlands in which stressful events were associated with increased relapses in relapsing–remitting MS [24]. Finally, in a one year study, 48 women with relapsing–remitting MS were divided into two groups, either receiving the anti-depressant drug escitalopram daily or continuing with MS treatment as usual, and stressful life events were documented weekly. The risk for relapse was 2.9 times higher for controls than for the escitalopram-treated patients [25]. Table 1 lists studies reporting stress–MS association in humans.

<table>
<thead>
<tr>
<th>Type of stress</th>
<th>n</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death of child</td>
<td>21,062 parents exposed 293,745 not exposed</td>
<td>The exposed parents had a significantly increased risk of MS for at least 8 years of follow-up.</td>
<td>[14]</td>
</tr>
<tr>
<td>Stressful life events (SLE) as reported weekly assessed with the Life Events and Difficulties Schedule</td>
<td>50</td>
<td>Relapses were more likely during at-risk periods following the events.</td>
<td>[15]</td>
</tr>
<tr>
<td>Diaries of SLE ranked according to the Holmes and Rahe Social Readjustment Rating Scale. Anxiety assessed with the Hamilton Rating Scale for anxiety</td>
<td>37</td>
<td>High levels of anxiety were strongly related with the advent of relapse in the following period.</td>
<td>[17]</td>
</tr>
<tr>
<td>Life stress</td>
<td>100 MS patients compared to controls</td>
<td>Significantly more MS patients than controls reported that they were under unusual stress in the 2 year period prior to onset age. MS patients described a greater number of SLE.</td>
<td>[20]</td>
</tr>
<tr>
<td>SLE assessed by the Psychiatric Epidemiology Research Interview</td>
<td>55</td>
<td>Patients who experienced qualitatively extreme events were 37 times more likely to relapse as those not exposed to such events. The proportion of MS patients who experienced marked life adversity in the year prior to onset of symptoms was significantly higher than for non-patients in the year before interview.</td>
<td>[22]</td>
</tr>
<tr>
<td>SLE</td>
<td>39 MS patients and 40 matched controls</td>
<td></td>
<td>[23]</td>
</tr>
<tr>
<td>Emotional stress</td>
<td>95 pairs of MS patients in relapse and remission</td>
<td></td>
<td>[21]</td>
</tr>
</tbody>
</table>

2.2. Animal studies

In mice with Theiler’s murine encephalomyelitis virus (TMEV) infection, a well characterized model of MS, restraint stress during early infection increased CNS lesion formation during the late phase [26]. Restraint stress in animals infected with TMEV had a global...
immunosuppressive effect on the immune response to infection [27], leading to increased mortality rates, decreased numbers of lymphocytes and increased numbers of neutrophils in the blood [28]. This paradigm also exacerbated acute CNS infection and subsequent demyelination [29]. Maternal separation (180 min/day) impaired host resistance during infection and prolonged TMEV [30]. A significant increase in the severity of neurological signs was noted along with pathological lesions of the spinal cord in stressed rats compared to non-stressed rats; treatment with alprazolam reversed the adverse effects of stress [31]. However, another study using Theiler's virus-induced demyelinating disease (TVID) in the resistant C57BL/6 mouse strain suggested that stress alone is not sufficient to overcome genetic resistance to TVID [32]. Acute restraint stress disrupted the BBB and substituted for diphtheria toxin permitting the development of myelin basic protein-induced EAE sooner in rats [33]. Administration of diazepam for 6 days, starting at day 6 or 11 after active induction of experimental allergic encephalomyelitis (EAE), an animal model for MS, led to a marked decrease of disease incidence, reduced histological signs associated with the disease, as well as cellular reactivity and antibody responses against the encephalitogenic MBP [34]. Table 2 lists studies showing stress–EAE association in animals.

3. Involvement of microglia and mast cells

Recent evidence indicates that microglia play an important role in the pathogenesis of MS. The most intense microglia infiltration has been observed in acute MS cases in which the acute stage inflammatory macrophage markers MRP14 and 27E10 were expressed [35]. It has also been shown that in a specific subtype of MS, where hypoxia-like lesions exist, microglial activation is prominent and precedes T-cell infiltration and demyelination [36]. Moreover, brain pathological findings from patients who died of MS exhibited extensive oligodendrocyte apoptosis and microglial activation in the relative absence of T-cells [37]. Actually, microglia act as antigen-presenting cells for naïve T-cells, thus expanding the number of encephalitogenic Th1 cells [38]. Moreover, microglia have the ability to produce glutamate and nitric oxide (NO), which have a direct effect on the death of neurons. NO also has a cytotoxic effect on the endothelium and contributes to the BBB disruption [38], which is known to precede many pathological or clinical symptoms of MS [39,40]. Furthermore, dying oligodendroglial cells recruit microglia which, in the presence of IFN-γ activation, induce contact-dependent oligodendroglial death [41]. Lastly, microglia are a rich source of reactive oxygen species (ROS), and various pro-inflammatory cytokines/chemokines and proteases [42].

On the other hand, microglia might have a role in the termination of the inflammatory reaction by suppressing lymphocyte reactivity through NO release [43]. In fact, a strong accumulation of CD 163(+) microglia with anti-inflammatory effects was found in acute active MS lesions and at the rim of chronic active lesions, possibly involved in the resolution of the inflammation [44]. Microglia also phagocytose apoptotic T-cells, even though this mechanism seems to be defective in MS [38].

It has been shown that in mice with EAE, microglial activation persists during the chronic phase of the disease, while T cell infiltrates are predominant during the acute phase of the disease [45]. Microglia participate in the pathogenesis of EAE not only by phagocytosing myelin and thus leading to demyelination [46], but also by releasing TNF-α, IL-1, IL-6 and chemokines, which promote inflammation during the course of the disease [47]. In fact, Lewis rats which are susceptible to EAE showed suppression of disease progression upon elimination of microglia [48].

MS is mediated primarily by brain infiltration of Th1 cells and macrophages [49], but Th2 processes typically associated with allergic reactions, which involve mast cells, are also implicated [50–52]. Mast cells have been reported in MS plaques [53] and could stimulate demyelination directly [54–56]. Clinical evidence supporting the involvement of brain mast cells in MS comes from the fact that the unique mast cell protease tryptase [57] and histamine [58] were elevated in the CSF of MS patients. Moreover, gene microarray analysis of MS plaques revealed increased expression of 5-lipooxygenase in acute lesions and the FcγRI receptor in chronic lesions, both of which are associated with mast cells [59,60]. Mast cells are also involved in Th17 maturation. Th17 cells are differentiated by the combined action of IL-6 and TGF-β to secrete IL-17 [61], shown to be critical for the pathogenesis of autoimmune diseases, including MS [61,62]. TNF-α and vasoactive intestinal peptide (VIP) can also induce Th17 maturation independently of IL-6 [63]. It is noteworthy that IL-6, TGF-β, TNF-α, and VIP can all be secreted by mast cells [64–66]. In fact, mast cells can even secrete IL-17 on their own [67]. Mast cell mediators can recruit and activate T cells, as well as permit them to enter the brain by disrupting the BBB [68]. Mast cells stimulated by FcγRII aggregation released TNF-α and activated T cells [69], but direct contact was also required [70]. Mast cell-derived leukotriene B4 promotes T cell migration [71]. Fig. 1 proposes a set of interactions between mast cells and T-cells.

In EAE, mast cells are required for optimal T cell responses [72], but can also degrade myelin directly [54,56]. Development of EAE has been shown to involve mast cell accumulation in the rat brain [73] that could be due to chemotactic activity elicited by RANTES [74] or MCP-1 [75] secreted from either glial cells or infiltrating leukocytes, EAE was attenuated and delayed in W/Wv mast-cell deficient mice [76], but was fully restored upon mast cell reconstitution even in the apparent absence of brain mast cell replenishment [77]. This effect apparently required mast cells outside the brain [78], especially in the meninges [79]. The authors concluded that brain mast cells are not important, but did not exclude the involvement of perivascular mast cells and their ability to regulate the permeability of BBB [80,81].

Interestingly, some recent data appear to suggest that mast cells may also have a protective effect on EAE development. Specifically, both activating and suppressing Fc receptors were recently shown to be expressed on mast cells and regulate EAE disease severity in

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Studies in animals reporting correlation between stress and EAE.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of stress</td>
<td>Outcome</td>
</tr>
<tr>
<td>Restrained stress (mice restrained in ventilated 60 ml plastic syringes in their home cages)</td>
<td>Inflammation and demyelination were significantly increased in spinal cords of stressed mice. Axonal degradation was increased in demyelinated areas in stressed mice.</td>
</tr>
<tr>
<td>Restrained stress</td>
<td>Stress had a global immunosuppressive effect on the immune response to infection. The adverse effects of stress were mimicked by dexamethasone, implicating a major role for glucocorticoids.</td>
</tr>
<tr>
<td>Restrained stress (mice placed in well ventilated restraining tubes, 2–3 cm internal diameter and 8 cm length)</td>
<td>Stress decreased both type 1 and type 2 responses to infection.</td>
</tr>
<tr>
<td>Maternal separation</td>
<td>Maternal separation 180 min/day impaired host resistance during infection and delayed the kinetics of viral clearance.</td>
</tr>
<tr>
<td>Restrained stress (mice placed in well ventilated restraining tubes, 2–3 cm internal diameter and 8 cm length)</td>
<td>Increased mortality rates were observed in restrained mice, which also developed higher CNS viral titers. Restrained-stressed mice developed decreased numbers of lymphocytes and increased numbers of neutrophils in the blood.</td>
</tr>
<tr>
<td>Social disruption stress</td>
<td>Social disruption stress applied prior to infection led to more severe disease course, with increased inflammation.</td>
</tr>
<tr>
<td>Restrained stress (mice restrained in ventilated 60 ml plastic syringes in their home cages)</td>
<td>Chronic restraint stress during early infection exacerbated acute CNS infection and the subsequent demyelination.</td>
</tr>
</tbody>
</table>
mice [82]. Myelin oligodendrocyte glycoprotein (MOG)\textsubscript{35-55}-induced EAE was exacerbated in mast-cell deficient Kit\textsuperscript{W-sh/W-sh} mice both at high and low antigen protocols, while Kit\textsuperscript{W/w} mice were protected when immunized with high, but not low doses of antigen [83]. In addition, when the mast-cell deficient mice were reconstituted with bone marrow derived mast cells, systemically, but not in the CNS, they still developed exacerbated EAE [83]. Any protective role against EAE may be exerted by Treg activation only in the brain. In fact, EAE in Kit\textsuperscript{W-sh/W-sh} mice developed earlier with more severe clinical and pathological symptoms; reconstitution with mast cells reduced susceptibility to the disease, and correlated with mast cell recruitment and T\textsubscript{reg} activation in the CNS [84]. Yet, mast cell-derived TNF exacerbated mortality during severe infection that was reduced in Kit\textsuperscript{w/w} mast-cell deficient mice [85]. Moreover, EAE development was reduced in TNF knockout mice [86], but became worse at a later date [86]. It is quite critical that results obtained from mouse models should be interpreted cautiously as they may not be readily applicable to humans [87].

4. Possible mechanisms to translate stress in MS risk

4.1. Stress and the HPA axis

Stress activates the hypothalamic–pituitary–adrenal (HPA) axis through the hypothalamic secretion of corticotropin-releasing hormone (CRH), which normally suppresses immune responses through the release of glucocorticoids from the adrenals [88]. In this context, it has been proposed that MS worsening with stress may be due to dysfunctional HPA axis because of reduced production of adrenal steroids. Impaired activation of CRH neurons was shown in those with active MS lesions in the hypothalamus, where the disease course was more severe [89]. Patients with secondary progressive MS also exhibit less cortisol production in response to CRH stimulation [90]. The absence of a normal cortisol response during systemic infection was reported in patients with MS suggesting impaired cortisol secretion and a reduced ability to control inflammation [91]. Despite the fact that clinical conditions characterized by overproduction of pro-inflammatory cytokines are associated with elevated circulating cortisol levels, this is not observed in MS patients [92]. Development of glucocorticoid resistance due to stress exposure may also result in increased CNS inflammation [93].

A temporal framework has also been proposed in order to explain the effects of stressful life events in patients with MS [94]. Specifically, acute stress might have a permissive effect on MS exacerbation by facilitating BBB breakdown, while chronic stress may lead to glucocorticoid resistance, making the immune cells less responsive to regulatory control by cortisol.

4.2. Stress, microglia and mast cells

CRH also has pro-inflammatory effects [95,96]. CRH affects brain microvessels directly [97] and activates mast cells [97,98] leading to increased BBB permeability which was absent in mast-cell deficient mice [99,98]. We have also reported that CRH and NT which are secreted under stress, synergistically stimulate mast cells leading to increased vascular permeability [100] and blood–brain barrier (BBB) disruption [101]. We further showed that NT stimulates mast cell secretion of vascular endothelial growth factor (VEGF) [102], which increases BBB permeability. NT also induces expression of CRH receptor-1 (CRHR-1) [103], activation of which by CRH increases the stimulation of human mast cells [104]. Animal experiments showed that acute stress led to BBB disruption in rats [105,106] and shortened the time of onset of EAE [33]. In fact, EAE could not develop in CRH knockout mice [107].

Stress activates microglia as well [108–110]. Specifically, exposure of rats to cold stress provoked morphological activation of microglia [108]. In addition, restraint stress combined with water immersion stress induced morphological activation of microglia in the thalamus, hypothalamus, hippocampus, substantia nigra, central gray, an effect that was significantly reduced in rats null for IL-18 [109].

CRH induces the proliferation and TNF-\(\alpha\) release by cultured rat microglial cells [111]. Microglia also express neurotensin (NT) receptor 3 (NTR3) leading to their proliferation and gene expression of macrophage inflammatory protein-2 (MIP-2), MCP-1, interleukin-1beta and TNF-\(\alpha\) [112]. SP receptors have been detected in both murine and human microglia, activation of which by SP led to the activation of NF-\(\kappa\)B transcriptional factor [42].

Human microglia have also been shown to produce SP [42], which is known to activate mast cells [113]. On the other hand, microglia respond to pro-inflammatory signals released from mast cells [114]. Mast cell tryptase induces microglial activation and pro-inflammatory mediator release of TNF-\(\alpha\), IL-6 and ROS [115]. Emerging evidence suggests that mast cell–microglial interactions play an important role in neuroinflammatory diseases [114]. Fig. 2 shows possible interactions between stress, microglia, mast cells and brain inflammation.

5. Conclusion

There have been important new treatment options for MS patients [116]. Moreover, recent evidence indicates that glatiramer acetate, in addition to having an immunomodulatory [117] and a neuroprotective effect [118], also decreases TNF-\(\alpha\) while increasing IL-10 secretion and promoting phagocytic activity of microglia [119]. However, addressing the effect of stress on MS is an entirely new treatment option. For instance, in a study of 121 patients with relapsing
MS, stress management therapy resulted in the reduction of the development of new MRI lesions [120]. Additionally, in a study of 62 patients with MS, it was shown that those who attended an 8-week stress-management program experienced a decreased number of weekly symptoms and mean intensity per symptom [121]. Moreover, publications using animal models reported that diazepam [34] or alprazolam [31] can decrease or reverse the clinical and histological signs associated with EAE. CRH antagonists [122] may also be useful since EAE could not develop in CRH knockout mice [107].

Microglia [36] and mast cells [80,123] have been considered as the next therapeutic targets for MS. However, there are no clinically available inhibitors of these cell types. Certain natural flavonoids, such as quercetin, luteolin and apigenin have anti-oxidant and anti-inflammatory effects [124]. These flavonoids also suppress TNF-α and IL-6 expression and release from microglia [125–127], as well as mast cell activation [128], and release of cytokines [129–131]. The flavonoids luteolin and quercetin decrease the amount of myelin phagocytosed by macrophages [132], as well as reduce EAE [133–135]. Luteolin also inhibits mast cell-dependent T cell activation [136]. Apigenin sensitizes activated human T cells to apoptosis and inhibits auto-antigen-presenting cells necessary for the expansion and activation of Th17 cells in lupus [137]. Propolis, a flavonoid-containing substance, inhibits IL-6 plus TGF-β-induced Th17 differentiation in vitro [138]. Luteolin inhibits activated peripheral blood mononuclear cells and had synergistic effect with IFN-β [139,140] prompting the suggestion that luteolin may be a reasonable adjuvant for MS treatment [139].

Authors’ contributions

All authors have read and approved the final manuscript. TCT designed and wrote most of the paper. AK and MA researched the literature and prepared the manuscript.

Disclosures

Dr. Theoharides is the inventor of US Patents No. 7906153 covering the use of flavonoids in the treatment of MS and No. 8268365 covering the use of flavonoids in the treatment of brain inflammation.

Conflicts

The authors report no conflict of interest.

Take-home messages

- Human and animal studies show a correlation between stress and relapses of multiple sclerosis.
- Microglia and mast cells are involved in the pathogenesis of multiple sclerosis.
- Stress activates microglia and mast cells through the release of the neuropeptide neurotensin and corticotropin-releasing hormone.
- Stress-induced activation of microglia and mast cells leads to BBB disruption and brain inflammation.
- Stress reduction as well as inhibition of microglial and mast cell activation can prove to be a useful adjunct to the current treatments of multiple sclerosis.

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Fig. 2. Diagrammatic representation of the effect of stress on brain inflammation and the proposed interactions between microglia and mast cells.


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