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The role of stress-response systems for the pathogenesis and progression of MS

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Disease progression in multiple sclerosis (MS) – an inflammatory demyelinating and neurodegenerative disease with a presumed T-cell driven autoimmune origin – has long been hypothesized to be associated with stress. However, this notion has only recently been supported by prospective clinical studies. Several clinical and molecular studies in MS and its animal models have recently shown disruptions in the communication between the immune system and the two major stress response systems, the hypothalamo–pituitary–adrenal (HPA) axis and the autonomic nervous system. Insensitivity to glucocorticoid and β-adrenergic modulation might be involved in overshooting inflammation in MS, whereas hyperactivity of the HPA axis has been linked to neurodegeneration and increased disability. Here, we integrate findings from molecular, cellular, experimental, clinical and epidemiological research to describe the involvement of stress response systems in MS pathogenesis and progression.

Stress and MS

Multiple sclerosis (MS) is an inflammatory, demyelinating and neurodegenerative disease with a presumed T-cell driven autoimmune origin (Box 1). Since the first description of MS, psychological stress has been suggested as a trigger for exacerbations [1] but, until recently, the clinical evidence for a causal relationship was weak.

A recent systematic meta-analysis of 14 prospective studies found a significant relationship between the occurrence of stressful life events and a higher risk for relapses (effect size of $d = 0.53$ [2]). This is within the same range as the effect sizes reported for the most common disease modifying medications [3], thus suggesting that the strength of the association between stress and MS exacerbation is consistent with effect sizes accepted by the field as clinically significant.

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Glossary

Astrocytes: a class of large (macroglial) cells in the CNS. Astrocytes are stellate shaped with many long processes that form the glial limitans and maintain the blood–brain barrier. Astrocytes perform several functions that are essential for neuronal activity, including glutamate metabolism, K^+ and H^+ buffering and water transport. They also crucially influence neuronal survival in response to injury by glial scar formation and provision of trophic support.

Combined dexamethasone-CRH test: this sensitive clinical test is used to assess feedback control of the HPA axis *in vivo* and is employed in psychiatric, endocrine and neurological research. After oral application of dexamethasone (Dex) the previous night, patients are injected with CRH to examine the efficiency of the feedback loop of the axis. Blood levels of ACTH and cortisol are measured in response to CRH injection. A strong response to CRH after dexamethasone pretreatment in rodents has been shown to reflect impaired negative feedback at the pituitary and higher levels. However, the exact mechanisms for abnormal results in this test in humans are unclear because counter-regulatory mechanisms might occur at each of the different levels of the feedback loop and a thorough examination of various HPA parameters is required to provide an explanation for the Dex-CRH test results.

Experimental autoimmune encephalomyelitis (EAE): rodents or non-human primates immunized with purified components of CNS myelin can be used as a model for MS. This approach is called 'actively-induced' EAE. Similarly, the disease can be induced by injecting sensitized immune cells ('adoptive transfer EAE'). Both approaches cause autoimmune attacks that mimic several pathological aspects of MS, including CNS inflammation, demyelination and axonal damage. However, no single model exists that mimics all the features of the human disease. The clinical course of the model can be acute relapsing, chronic relapsing or chronic progressive, depending on species, strain and peptide used.

GC-sensitivity: the sensitivity of immune cells for inhibition by glucocorticoids (GCs) can be tested *in vitro*. Immune cells are cultured with stimulants (such as phytohemagglutinin, PHA) in the presence and absence of GCs to evaluate the inhibitory effect on cytokine production capacity or cell proliferation.

Hypothalamo-pituitary-adrenal (HPA) axis: this neuroendocrine axis is controlled by the hypothalamus, which receives input from higher cortical areas and other brain regions, including the limbic system. Activation of the axis leads to corticotropin releasing hormone (CRH) release from the paraventricular nucleus (PVN) of the hypothalamus, which in turn triggers the release of adrenocorticotropic hormone (ACTH) from the pituitary. This ultimately induces glucocorticoid release from the adrenals. The hormones secreted by the HPA axis have potent effects on immune function and other target tissues and are under a tight negative feedback control at several levels. Among the main activators for the HPA axis are psychological or physiological stressors, including inflammatory cytokines.

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Box 1. Overview of multiple sclerosis pathology and clinical course

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) affecting ~2.5 million people worldwide.

Pathogenesis

MS is a heterogeneous inflammatory, demyelinating and degenerative disease of a presumed Th1-autoimmune origin that occurs in genetically susceptible individuals [57]. The proposed pathogenetic mechanism is peripheral activation of autoreactive $CD4^+$ T cells targeting proteins of the myelin sheath of neurons. Upon activation, these cells enter the CNS through the blood–brain barrier, recognize myelin antigens and initiate a chronic inflammatory cascade that results in demyelination of axons, mainly by macrophages [66]. Different subtypes with varying involvement of humoral (antibodies and complement) and cellular mechanisms, as well as a primary oligodendroglial degeneration and apoptosis, have also been proposed [67].

The pathological hallmark is the demyelinated plaque, which consists of well-demarcated areas characterized by loss of myelin and formation of astrocytic scars. However, it is becoming increasingly clear that axonal loss that mainly occurs later in the disease process might be the major determinant for long-term, permanent disability. It is unclear whether all neurodegeneration is directly related to acute inflammation because diffuse axonal damage could occur separately from pathological lesions.

Clinical course

Clinically, there are two manifestations of the disease: relapses and progression. Relapses are sudden (within 24 h) increases in symptoms that remit partially or fully in a matter of weeks or months. Progression refers to a slower, steadier increase in symptoms in the absence of relapses. Relapses are typically accompanied by acute inflammatory lesions whereas progression is thought to be driven by permanent neuronal damage.

In most cases, MS begins with a relapsing-remitting (RR) course characterized by exacerbations with quiescence between relapses. In the later stages, most patients develop a secondary progressive course (SPMS), marked by decreasing numbers or cessation of relapses and increased progression. The prognostic relevance of relapses is not clear but a high rate of severe relapses in the beginning is regarded a predictor of worse outcome. A small proportion of patients have a primary progressive course (PPMS) from the onset.

***In vivo* imaging of multiple sclerosis pathology**

In clinical studies, blood–brain barrier leakage, a marker of acute inflammation in the CNS, can be tracked *in vivo* by gadolinium enhancement on a T1-weighted MRI scan (Gd+ lesions). Hyperintense lesions on T2-weighted or fluid-attenuated inversion recovery (FLAIR) scans are considered the 'footprint' of inflammation and might reflect the chronic 'burden of disease'. Sequences to monitor tissue loss and neuronal damage include atrophy measures and 'black holes' (hypointense lesions) on T1-weighted scans and neuronal markers on magnetic resonance spectroscopy (MRS). A recent comprehensive overview of *in vivo* assessment of MS pathology with MRI can be found in [68].

However, in 1999 a task force of the American Academy of Neurology (http://www.aan.com) stated that no conclusion could be drawn before a biological model could be established and suggested that the use of gadolinium enhancing magnetic resonance imaging (MRI) (Gd+, a marker of acute focal brain inflammation; Box 1) could provide such confirmation [4]. The first such data were published in 2000, showing that the occurrence of stressful life events was associated with a significantly increased risk of a new Gd+ MRI brain lesion eight weeks later [5]. Thus, the literature to date has supported an association between the occurrence of stressful life events and the subsequent development of MS disease activity marked by both clinical exacerbation and a neuroimaging marker of brain inflammation. However, a clearly articulated model of the role of the primary systems involved in the regulation of stressful life events is lacking.

It is widely accepted that activation of the two major stress response systems, the hypothalamo–pituitary–adrenal (HPA) axis (see Glossary) and the autonomic nervous system (ANS) (Box 2) exert potent effects on immune cells [6]. These two response systems are closely linked and interact at multiple levels, modulating each others effects on the immune system and other target tissue (Box 2) Here, we review the animal and human data on the HPA and ANS with the goal of articulating a biological model that hypothesizes a role of neuro–endocrine–immune networks in the pathogenesis and progression of MS.

The HPA axis

The HPA axis is protective in EAE

Animal data indicate that a functional HPA hyporesponsiveness might have a role in MS susceptibility and severity. In all experimental autoimmune encephalomyelitis (EAE) models, the HPA axis is activated during clinical disease with declining corticosterone plasma levels parallel to the resolution of the disease [7–9]. In Lewis rats, which have a genetically determined HPA hyporesponsiveness, the impaired HPA axis response contributes to higher susceptibility to EAE [10]. Also, treatment with glucocorticoids (GC) reduces the rate and severity of EAE after adrenalectomy [11].

Interestingly, a reduction in HPA axis responsiveness to inflammatory stimuli might occur during the course of chronic relapsing remitting (CR-) EAE: whereas the initial relapse in a chronic relapsing EAE model was accompanied by an adequate HPA response, subsequent relapses were associated with lower corticosterone increases. Such an insufficient HPA response to inflammatory stimuli in EAE was shown to predict deleterious outcome significantly [12]. However, disease progression is inhibited if the circulating corticosterone level is maintained at levels seen during the initial phase of disease, supporting the concept that low corticosterone is causal to more severe EAE. Thus, the functional status of the HPA axis seems to have a relevant role in the control of EAE.

HPA hyperactivity is associated with disease severity in MS

In contrast to early EAE studies postulating a hyporesponsive HPA axis as a predisposing factor for disease susceptibility and severity, most clinical studies generally support the notion of HPA hyperactivity in MS. Basal plasma levels of cortisol and adrenocorticotropic hormone ACTH were found to be elevated [13] and adrenals were enlarged in MS patients [14]. Furthermore, post-mortem studies reported an increased number and activity of corticotropin releasing hormone (CRH)-expressing neurons co-localizing vasopressin in the hypothalamus of MS patients compared to controls [15,16]. Findings with the dexamethasone suppression test yielded contradictory

Box 2. The autonomic nervous system and its relation to the immune system and the HPA axis

The hypothalamo–pituitary–adrenal (HPA) axis and the autonomic nervous system (ANS) are the two major stress-response systems.

Autonomic nervous system

The ANS is divided into two subsystems, the sympathetic (SNS) and the parasympathetic (PNS), which work in tandem, either in a synergistic or an antagonistic way. The sympathetic fibres run down the spinal cord and branch off to ganglia situated next to the vertebral column from which they innervate major organs. The parasympathic nerve descends directly from the brainstem and innervates most peripheral organs. Activation of the SNS ultimately leads to release of epinephrine and norepinephrine from the adrenals into the blood stream. Norepinephrine and NPY are further directly released from nerve endings in lymphoid tissue. The main transmitter released from the parasympathetic nervous system is acethylcholine (ACh).

Interface with the immune system

The adrenergic SNS modulates local immune responses. Noradrenergic sympathetic nerve fibres run from the CNS to primary and secondary lymphoid organs, such as the thymus, spleen and lymph nodes. The primary neurotransmitter released from sympathetic nerves, norepinephrine, exerts its effect at the target tissues through its receptor, the adrenergic receptor. Numerous cells of the immune system, including lymphocytes and macrophages, express adrenoreceptors. These are G-protein coupled receptors that can be divided into two subgroups – the α- and β-adrenergic receptors. Expression of α- and β-adrenergic receptors on T and B lymphocytes, neutrophils, mononuclear cells and NK cells has been described. Activation of β_2-adrenergic receptors results in an increase in cyclic AMP concentrations, which can modulate cytokine expression (e.g. decreasing TNF-α and increasing IL-8). Recent data suggest that norepinephrine protects against progression of autoimmune disease by modulating B cell function and inhibiting Th1-like responses. If α-adrenergic receptors are present on immune cells, then activation of these receptors will lead to the activation of a different signalling cascade and the activation of MAP kinases. Activation of this cascade induces a shift towards Th1-like cytokine patterns [40]. Other transmitters of the sympathetic nervous system, such as neuropeptide Y (NPY), might modulate the effects of catecholamines on immune function [62]. Parasympathetic modulation of the immune system is less well studied; however, ACh receptors are expressed on immune cells and the vagal nerve can exert powerful anti-inflammatory effects [69].

SNS–HPA axis interaction

Functionally, the HPA axis and the SNS participate in a positive feedback loop so that activation of one activates the other [70]. Both systems can be activated by many of the same neurochemical mediators, and glucocorticoids and catecholamines can synergistically suppress cellular and potentiate humoral immunity. Interactions on the central level include projections from neurons in the paraventricular nucleus (PVN) of the hypothalamus (the control centre of the HPA axis) to the sympathetic system in the hindbrain.

results with diminished [17] or normal cortisol-suppression after dexamethasone [18,19].

Correlation with inflammation markers

Using sensitive stimulation tests, Wei *et al.* [18] found higher responses of ACTH to CRH in MS patients with enhancing lesions, whereas cortisol was lower after administration of CRH in those patients, most of whom were in relapse. Similarly, Fassbender *et al.* [20] showed a correlation of cortisol hyper-responsiveness [as measured by the dexamethasone (Dex)-CRH suppression test] with inflammatory activity in the brain, as assessed by white cell counts in the CSF and Gd-enhancing lesions on MRI in a sample of relapsing-remitting MS (RRMS) (Box 1). It is interesting to note that in a sample of both RRMS patients and secondary progressive MS patients (SPMS), Schumann *et al.* [21] found a negative correlation of a hyper-responsive Dex-CRH test with Gd-enhancing lesions and no correlation with inflammatory markers in blood and CSF. These studies suggest that a positive correlation between inflammation markers and HPA axis activation can only be found during relapses and not in remission or the progressive phase of the disease.

Correlation with atrophy, disability and progression

Schumann *et al.* [21] have reported a positive correlation of HPA activity in the Dex-CRH test and an MRI measure of global atrophy. Subsequently, Then Bergh *et al.* [22] and Heesen *et al.* [23] have both shown HPA hyperactivity in patients in the progressive disease phase (SPMS, PPMS), which correlated with disability. The latter study also described a correlation with cognitive dysfunction, an association that was replicated in another patient sample [24].

In the first longitudinal study, Gold *et al.* [25] found that Dex-CRH hyperactivity significantly predicted disease progression [as measured by an increase of at least 1 point on the expanded disability status scale (EDSS), the most commonly used clinical outcome measure in MS] over a three year follow-up period. This indicates that results from the Dex-CRH tests could be of prognostic value in MS.

The role for the HPA axis in MS

Hypothesis 1: glucocorticoid (GC)-insensitive immune cells enable inflammation overshoot during relapses

There is emerging evidence that GC-sensitivity of immune cells is impaired in MS. DeRijk *et al.* [26] showed that immune cells obtained from RRMS patients were less sensitive to steroid-induced suppression of interleukin-6 (IL-6) production than cells from healthy controls. GC-sensitivity of cells from patients with progressive disease was not significantly different from controls. The authors also report a trend of correlation of *in vitro* GC resistance and disability in a small subsample with longitudinal clinical data. Recently, van Winsen *et al.* [27] confirmed a reduced GC sensitivity to suppress lipopolysaccharide (LPS)-induced tumour necrosis factor-α (TNF-α) production in whole blood cultures in a large group of RRMS patients, compared to healthy controls. In chronic disease courses this altered GC sensitivity was less obvious. Apparently, GC insensitivity is specific to cytokine production because Wei *et al.* [18] found no altered biological effects of GC on blood lymphocyte proliferation in MS.

Mechanisms for GC-resistance in MS have not yet been elucidated. Whereas the binding capacity of GC receptors expressed by peripheral blood mononuclear cells (PBMCs) is normal in MS, there appears to be a decoupling of GC-receptor affinity on PBMCs and HPA axis activity, as measured by the Dex-CRH test [28].

We hypothesize that GC insensitivity could be one pathway that is linked to the development of chronic inflammation in the CNS, as seen in RRMS patients. As described earlier, during relapses the HPA axis seems to respond to inflammation. This response, however, might be insufficient to control inflammation due to decreased GC sensitivity in the immune system. Also, as mentioned previously, insufficient HPA responses to inflammatory stimuli are associated with faster disease progression in CR-EAE [12]. Although the HPA axis response to inflammatory stimuli in MS has not been studied directly, reduced cortisol responses to sepsis [29,30] and the animal data suggest that inadequate HPA responses to inflammatory stimuli could further promote inflammation in early disease stages.

Hypothesis 2: HPA axis hyperactivity is a marker of accumulating neurodegeneration

We hypothesize that HPA axis hyperactivity in later MS is most probably the result of neurodegeneration in brain areas involved in glucocorticoid feedback control. For example, damage in projections to the hypothalamus might disturb inhibitory signals to the HPA axis and thus account for the hyperactive HPA axis. Other candidate brain areas controlling the HPA axis include the hippocampus and prefrontal regions. This is supported by the correlations of HPA axis hyperactivity with cognitive impairment in MS [23] because the hippocampus and the pre-frontal region have pivotal roles in memory, attention, information processing and executive function. Further support comes from Schumann *et al.* [21], who showed a correlation of decreased HPA feedback (as measured by the Dex-CRH test) and a measure of global atrophy on MRI.

Axonal transection and neurodegeneration are thought to cause permanent physical impairment in MS [31]. An association of HPA axis activity and increasing neuronal damage could thus explain its prognostic value for clinically significant increases in disability [25]. We hypothesize that the ongoing neurodegeneration causes a steady decline of HPA axis feedback control (thus resulting in increased HPA responses). At some point, the HPA hyperactivity might seize control of the inflammation. Clinically, this point would be expected to coincide with the transition from relapsing-remitting to secondary progressive disease with less evidence of focal inflammation but accumulating neurodegeneration. However, to date, this hypothesis has not been tested empirically (Box 3).

Patients with RRMS, SPMS and PPMS typically seen in clinical populations do not reflect the entire spectrum of MS. Although clinical studies suggest that HPA hyperactivity might be associated with neurodegeneration during later stages of the disease, there is evidence for HPA hyporeactivity having a potential role in severe MS. In a post-mortem study, Huitinga *et al.* [32] identified a special subgroup of patients with HPA deficiency, which might be involved in the pathogenesis of severe MS. In their study, which included relatively young MS patients with severe MS that came to autopsy, high inflammatory activity was associated with lower CRH mRNA expression in the hypothalamus and shorter time to death (i.e. more severe disease). These patients might experience inflammation unrestricted by endogenous glucocorticoids.

Box 3. Outstanding questions

Research on the endocrine–immune network in MS can help to elucidate pathologically relevant mechanisms. Further research is necessary regarding the hypothalamo–pituitary–adrenal (HPA) axis, the autonomic nervous system (ANS) and the interactions between the two.

HPA axis

(i) Is low HPA-axis responsivity in early disease stages, especially to inflammatory stimuli, a predictor for more CNS inflammation (Gd-enhancing lesions on MRI) and a worse disease course of MS?
*Strategy:*Challenging the HPA axis of newly diagnosed MS patients or patients with clinically isolated syndrome (CIS) using mild inflammatory stimuli (e.g. low dose endotoxin) and monitor the disease course longitudinally with serial MRI and clinical outcome measures.
(ii) Is low glucocorticoid (GC) sensitivity of immune cells in early stages of MS a predictor for more CNS inflammation and relapses?
Strategy: Clinical and MRI follow-up assessment of RRMS patients with and without marked GC sensitivity at early disease stages.
(iii) What are the underlying mechanisms of GC insensitivity in MS? Which cell populations are affected (e.g. T cells, monocytes)?
Strategy: Investigate receptor polymorphisms in MS patients that potentially affect sensitivity, as well as molecular studies of receptor signalling (e.g. translocation to nucleus, transcription factors) in immune cell subpopulations obtained from MS patients to elucidate possible disturbances in the signalling cascade.
(iv) Does axonal damage relate to high HPA-axis responsivity and the conversion from relapsing-remitting to secondary progressive MS?
Strategy: Cross-sectional studies employing MRI and MRS (Box 1) to correlated HPA response (Dex-CRH test) with measures of atrophy and axonal damage ('black holes' on MRI, neuronal markers in MRS). Longitudinal studies of RRMS patients likely to progress to SPMS (EDSS around 3) to examine HPA status changes during the transition phase.

Autonomic nervous system

(i) To what extent does intracellular dysfunction of adrenergic receptors relate to a worse disease course of MS?
Strategy: Cross-sectional and longitudinal examination of clinical and paraclinical (e.g. MRI) measures of disease severity and progression in MS patients with insensitive adrenergic receptors and disturbed intracellular signalling.
(ii) Do β mimetic agents alter the disease course of MS?
Strategy: Placebo-controlled pilot trials to treat MS patients with β-adrenergic agonists (terbutaline, salbutamol) over an extended period of time (minimum 1 month) to examine changes of *in vivo* immune responses. Longer treatment period to test clinical efficacy.
(iii) To what extent are other neurotransmitters of the SNS involved in immune modulation of MS (i.e. NPY)?
Strategy: Examine the immunomodulatory role of NPY on immune cells *in vitro* obtained from MS patients and correlate with clinical markers.
(iv) Is the parasympathetic modulation of the immune system disturbed in MS (i.e. the inflammatory reflex) and what is the therapeutic potential of vagal stimulation in MS?
Strategy: Examine the inflammatory responses after vagal stimulation in animal models of MS.

The autonomic nervous system

The ANS is protective in EAE

Chemical sympathectomy with 6-hydroxydopamine (6-OHDA) at birth led to a more severe course of the disease in an EAE rat model, as well as in adoptive

transferred EAE [33,34]. Based on these findings, several groups have studied the influence of pharmacological stimulation or blocking of the receptors that bind neurotransmitters secreted from the sympathetic nervous system (SNS; adrenergic receptors, Box 2). Early studies showed that classical EAE, as well as adoptive transferred EAE, in Lewis rats could be prevented by prazosin treatment – an α_1-adrenergic receptor antagonist – whereas α_2-agonist or β-antagonist (Box 2) treatment exacerbated the disease [35,36]. The β-adrenergic agonist isoproterenol and, to a lesser extent, terbutaline were both able to protect Lewis rats from monophasic EAE [37]. This protective effect was also demonstrated in a relapsing model in the Lewis rat [38]. Interestingly, similar results were obtained when treatment was started after the disease was already established. These studies suggest that activation of β-adrenergic pathways can ameliorate EAE.

Numerous studies have been conducted to investigate the relevance of α- and β-adrenergic receptors on immune cells and their effect on immune responses *in vitro* and *in vivo* (Box 2). Apart from suppressing B-cell and T-cell functions, β-adrenergic agonists decrease the phagocytic activity of macrophages (thus possibly decreasing demyelination) and inhibit interferon-γ (IFN-γ) induced MHC II expression (thus suppressing antigen presentation) and TNF-α secretion. Beta$_2$-adrenergic treatment induces a Th1–Th2 shift [39] because it inhibits IL-12, IFN-γ and TNF-α and stimulates IL-10. By contrast, α_2-adrenergic treatment stimulates Th1-like cytokine patterns [40]. These studies clearly show the immunomodulatory properties of adrenergic activation.

Autonomic regulation in MS

Well-known manifestations of autonomic dysfunction in MS include bladder, bowel and sexual dysfunction. Clinically overt cardiovascular dysfunction is rarely present but several clinical studies found subclinical involvement of the cardiovascular autonomic system in MS patients (reviewed in Ref. [41]).

More importantly, some studies suggest alterations of catecholamine levels in MS. Whereas Karaszweski *et al.* [42] found elevated norepinephrine (NE) serum levels in chronic progressive MS patients, Flachenecker *et al.* [43] reported reduced NE levels in clinically active RR MS patients (more than two relapses in the preceding two years).

Rajda *et al.* [44] described higher intracellular epinephrine levels in lymphocytes obtained from MS patients with their first attack, compared to RR patients in remission, whereas NE levels were significantly lower in RR patients. By contrast, Consentino *et al.* [45] showed higher intracellular NE levels in PBMCs of patients compared to controls. These results are in line with a disturbed autonomic regulation in MS.

ANS involvement in MS

Hypothesis 1: functional disturbance of ANS immunoregulation is involved in immune activation and infiltration of the CNS

Recently, it has been hypothesized that insufficient control of the immune response by the ANS might be relevant in the development of Th1 autoimmune diseases. Sympathetic modulators, as well as a fast-acting parasympathetic connection that detects the presence of inflammatory stimuli [46], appear to have a key role in maintaining a balanced immune system. It is now becoming clear that in Th1-driven autoimmunity, this communication can be disrupted, leading to the hypothesis that many of these diseases might actually be diseases of autonomic dysfunction [47].

Clinical studies in MS have provided evidence in support of autonomic dysfunction. For example, adrenergic receptor expression and function are altered in immune cells obtained from MS patients. Karaszewski *et al.* [48] and Zoukos *et al.* [49] both found an increased number of β-adrenergic receptors on lymphocytes in MS. Higher expression of β-receptors on CD8+ cells has been reported in progressive MS but not in RR or stable MS patients [50]. Zoukos *et al.* [51,52] found significant correlations of increased receptor density in RR MS patients with an ongoing relapse, new or enhancing MRI lesions and IL-2 receptor expression on PBMCs. They postulate that increased β-receptor density on immune cells could represent a counter-regulatory mechanism due to sympathetic denervation to control immune responses shifted towards a Th1-like pattern.

Despite increased receptor density, Heesen *et al.* [53] have shown no response in cytokine production capacity (IL-10 and IL-12) to *in vivo* administration of terbutaline (a β_2-adrenergic agonist) in MS patients compared to healthy controls, who responded strongly. Similarly, a recent study by Haerter *et al.* [54] showed a reduced terbutaline-induced inhibition of IFN-γ production by immune cells in rats with relapsing EAE.

Recent evidence indicates that a reduction of G-protein coupled receptor kinase (GRK) is implicated in the intracellular signalling dysfunction of adrenergic receptors in MS. It has been shown that leucocytes obtained from MS patients show a diminished expression of GRK2 [55,56] and an increased isoproterenol-induced cAMP-accumulation [55] without functional output. Taken together, these results suggest that the upregulation of receptor expression as seen in MS is inefficient in restoring the adrenergic sensitivity of immune cells because of a disturbed intracellular signalling cascade. Animal models suggest that reduced levels of GRK2 cause earlier onset of EAE accompanied by CNS infiltration of T cells and macrophages [56]. A defect in adrenergic signal transduction could thus lead to Th1-like immune responses, as well as enhanced recruitment of activated immune cells into the CNS, both of which are thought to have an important role in MS pathogenesis [57]. Therefore, the intracellular signalling disruption appears to be pathologically relevant.

Hypothesis 2: central lack of adrenoreceptors alters astrocytic protective functions

The distribution of β-adrenergic receptors in the CNS could be of relevance in MS. In contrast to the increased peripheral receptor density, De Keyser *et al.* [58] report a lack of β_2-adrenergic receptors on astrocytes within the brain of MS patients.

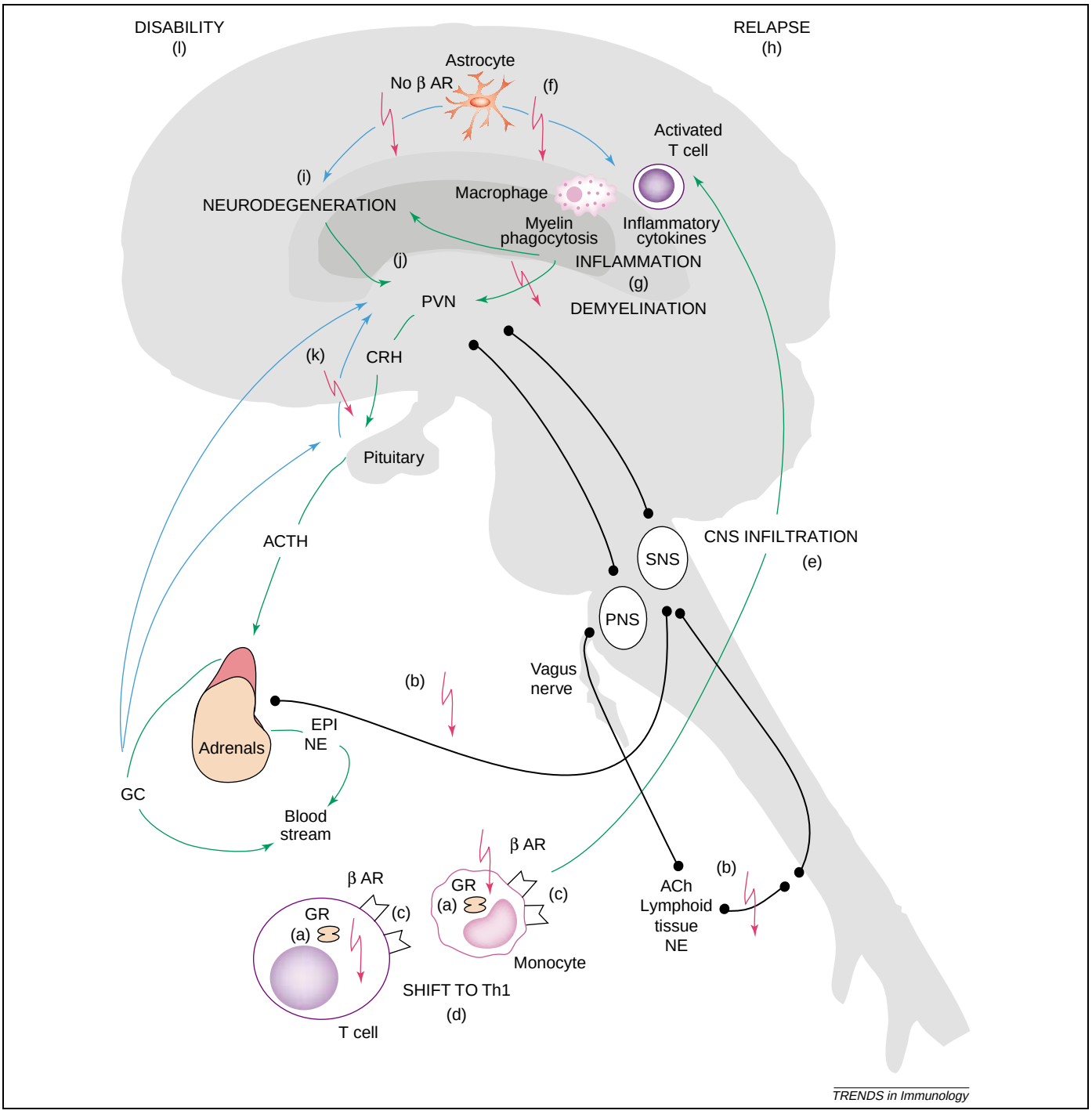

Figure 1. Schematic view of a biological model to describe the involvement of the hypothalamo–pituitary–adrenal (HPA) axis and the autonomic nervous system (ANS), which comprise the sympathetic (SNS) and parasympathetic nervous system (PNS), in pathological processes of multiple sclerosis (MS). The HPA axis and the SNS are activated by cytokines and other mediators released during inflammatory episodes of the disease. This activation results in the release of glucocorticoids (GC) from the adrenal cortex, as well as epinephrine (EPI) and norepinephrine (NE) from the adrenal medulla and NE from the sympathetic nerve endings. GCs bind to the intracellular GC receptor of immune cells and inhibit proinflammatory responses in healthy individuals. In MS, this inhibitory pathway is disturbed **(a)**, thus enabling a shift towards Th1-like immune responses. The response to inflammation by the SNS appears to be lowered **(b)**, as indicated by lower levels of NE during active relapsing-remitting MS (RRMS) compared to later progressive stages. Furthermore, despite the higher number of β-adrenergic receptors (βAR) on immune cells obtained from MS patients, the modulating influence of NE via this pathway is disturbed due to impaired intracellular signalling **(c)**. This insensitivity of immune cells to GCs and β-adrenergic modulation in combination with SNS (and possibly HPA) hyporesponse to inflammation is hypothesized to promote Th1-like responses **(d)**, as well as CNS infiltration by macrophages and T cells **(e)** during relapses. Within the CNS, βARs are not expressed on astrocytes, which disrupts several protective functions, such as adhesion molecule downregulation and inhibition of proinflammatory cytokine release **(f)**. This could be related to unrestricted inflammation and demyelination **(g)**. Clinically, this is linked to the occurrence of relapses **(h)**. A decreased glycogen synthesis, lactate supply and production of trophic factors from astrocytes, which is also mediated through β-adrenergic regulation, might promote neurodegeneration in MS patients **(i)**. Neurodegeneration is linked to HPA axis hyperactivity **(j)** by impairing the central feedback loop of the axis **(k)**. Neurodegeneration is thought to drive the development of irreversible disability **(l)** and possibly HPA-hyporesponse to inflammation. Blue arrows (inhibition), green arrows (activation), black lines (innervation), red arrows (disruption seen in MS). Abbreviations: ACTH, adrenocorticotropic hormone; CRH, corticotropin releasing hormone; GC, glucocorticoid; GR, glucocorticoid receptor; EPI, epinephrine; NE, norepinephrine; PVN, paraventricular nucleus of the hypothalamus; βAR, β-adrenergic receptors.

Astrocytic functions mediated by activation of β-adrenergic receptors include immunomodulatory functions, such as adhesion molecule downregulation and inhibition of proinflammatory cytokine release and possibly MHC II expression, as well as glycogen synthesis, lactate supply and production of trophic factors (reviewed in Ref. [59]).

In combination, disturbed AR signalling, decreased axonal energy metabolism and CNS immunomodulation could have an important role in the immune cell activation, migration into the CNS, and the induction of inflammatory demyelination and axonal degeneration in MS. Thus, the described absence of β-adrenergic receptors on astrocytes might be implicated in decreased immunosuppression and impaired neuroprotection in MS.

Hypothesis 3: prolonged treatment with adrenergic agonists might rescue immune control

Despite the insensitivity of peripheral immune cells to acute adrenergic stimulation, prolonged treatment with adrenergic agonists might induce a potentially beneficial shift in cytokine patterns. In a study with SPMS patients, Makhlouf *et al.* [60] showed that, after 14 days of treatment with salbutamol, the *in vitro* IL-12 production of monocytes and dendritic cells was decreased significantly compared to baseline, with persisting effects for at least one week post-treatment. Furthermore, they reported an increase of Th2 cytokines [61]. Based on these findings, a randomized trial of add-on treatment of salbutamol to glatiramer acetate is under progress (see http://www.clinicaltrials.gov).

To date, it is unclear how this reinstitution of adrenergic signal transduction is achieved. Future studies should examine β-adrenergic receptor expression and intracellular signalling cascades under β-agonist treatment in MS patients.

It is important to recognize that, in addition to catecholamines, the ANS secretes other immunomodulators, including neuropeptide Y (NPY), which differentially regulates the effects of epinephrine and norepinephrine on immune function, (see Ref. [62] for a recent review of the role of NPY in Th1 autoimmunity). Thus, understanding the functional complexity of humoral crosstalk between the SNS and the immune system requires assessment of the interactions of catecholamines and transmitters, such as NPY, which might also be instrumental in developing strategies to overcome β-adrenergic signalling disruption in EAE and MS.

Conclusion and future perspectives

There is increasing evidence that stressful life events correlate with exacerbations in MS. We furthermore have substantial evidence that alterations in the two major stress systems can be observed in MS. We are beginning to understand how these alterations might be associated with pathological processes of the disease (Figure 1).

Based on the evidence reviewed here, an insensitivity of inflammatory cells to adrenergic and GC modulation might be relevant for the occurrence of relapses in RRMS. It is, to date, unclear if and how disruptions in the neuroendocrine–immune network might affect susceptibility to the disease; however, studies suggest that HPA and ANS dysfunction might mediate disease progression. As discussed earlier, the development of permanent disability presumably due to irreversible neuronal damage in later stages of the disease (SPMS) could be linked to a disturbed feedback of the HPA axis. The breakdown of astrocytic support for affected neurons due to the loss of β-adrenergic stimulation is an intriguing hypothesis, possibly linking adrenergic dysregulation to neurodegeneration in MS, and the relevance of this process for the development of disability should be explored further. Unfortunately, little is known about the involvement of the stress response systems in PPMS. It is, however, interesting to note that HPA axis dysregulation appears to be frequent in this subgroup [22], which is characterized by progressive increases in disability in the absence of relapses.

These findings clearly warrant further investigation of the endocrine-immune network in MS (Box 3). There is also evidence that stress system dysregulation, although possibly following a different pattern, is also present in other inflammatory or autoimmune diseases, such as rheumatoid arthritis [63] and lupus erythematosus [64]. Interdisciplinary research will potentially yield information on how the interactive network between the SNS, the HPA axis and the immune system specifically and differentially affects autoimmune processes. For example, it is interesting to note that HPA axis hyperactivity is common in late MS but is not always seen in other chronic inflammatory diseases [65], thus pointing to an important role of the CNS involvement in the MS neuro–endocrine–immune network. We believe that this research can enhance our understanding of MS pathology and lead to the development of new progression markers, as well as potentially highlighting new avenues for therapeutic intervention targets.

These findings might also help to better understand the association between stressful life events and disease exacerbations; however, at this time, it remains difficult to draw direct conclusions about how alterations in the HPA axis and the SNS might be implicated in this association. Based on our biological model, one would postulate that the control of immune function by the major stress systems is impaired in MS, which might render the endogenous inhibitory signals inefficient against overshooting inflammation. This needs to be tested in prospective epidemiological studies that employ endocrine, immunological and MRI activity markers.

Future research should specifically address which of the reviewed disturbances in the endocrine-immune network are implicated in disease susceptibility, which ones are purely epiphenomena of increasing damage over the course of the disease, and which ones could be used to monitor relevant pathological processes.

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