Abstract: Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating disease of the CNS, most frequently starting with a series of bouts, each followed by complete remission and then a secondary, progressive phase during which the neurological deficit increases steadily. The underlying molecular mechanisms responsible for disease progression are still unclear. Herein, we demonstrate that high mobility group box chromosomal protein 1 (HMGB1), a DNA-binding protein with proinflammatory properties, is evident in active lesions of MS and experimental autoimmune encephalomyelitis (EAE) and that HMGB1 levels correlate with active inflammation. Furthermore, the expression of the innate HMGB1 receptors—receptor for advanced glycation end products (RAGE), Toll-like receptors RAGE, TLR2, and TLR4—was also highly increased in MS and rodent EAE. Additionally, in vitro activation of rodent CNS-derived microglia and bone marrow-derived macrophages demonstrated that microglia were equally as capable as macrophages of translocating HMGB1 following LPS/IFN-γ stimulation. Significant expression of HMGB1 and its receptors on accumulating activated macrophages and resident microglia may thus provide a positive feedback loop that amplifies the inflammatory response during MS and EAE pathogenesis. J. Leukoc. Biol. 84: 000–000; 2008.

Key Words: autoimmunity · DAMP · pathology · Toll-like receptor (TLR) · innate immunity

INTRODUCTION

High mobility group box chromosomal protein 1 (HMGB1) is a nuclear protein, which when released from cells, is able to act as a cytokine, mediating cell migration [1], differentiation [2], and tumorigenesis [3], as well as being implicated in the pathogenesis of several inflammatory diseases [4, 5]. HMGB1 can be actively released from stimulated monocytes and macrophages [6] or passively released by necrotic but not apoptotic cells [7]. Interestingly, HMGB1 and several proinflammatory cytokines mutually induce their release from macrophages/monocytes, thus forming a proinflammatory loop [8, 9]. Furthermore, HMGB1 is released in the brain following cytokine stimulation and ischemia [10] and elicits an inflammatory response when it is administered intracerebroventricularly [11].

The receptors implicated in HMGB1 binding are the receptor for advanced glycation end products (RAGE) [8, 12, 13] and the innate TLR2 and -4 [14, 15].

The present study focused on assessing the involvement of HMGB1 in the inflammatory process of multiple sclerosis (MS) and its animal model counterpart experimental autoimmune encephalomyelitis (EAE). We demonstrate that macrophages and microglia are major sources of HMGB1 in MS lesions as well as in rodent EAE lesions. We also demonstrate that an elevated HMGB1 expression correlates with an increased expression of the HMGB1-binding receptors RAGE, TLR2, and TLR4 in tissues and importantly, also in cerebrospinal fluid (CSF) cells. In conclusion, our data indicate that HMGB1 and its receptors may amplify inflammatory responses and thereby promote the neuroinflammatory processes in MS and EAE.

MATERIALS AND METHODS

Immunohistochemistry of MS autopsy tissues

This study was performed using archival autopsies from MS cases (collected at the Center for Brain Research, Medical University of Vienna, Austria), which were formalin-fixed and paraffin-embedded. The material included 45 cases of MS (average age: 45.5±11.7 years), seven cases of age-matched controls (average age: 39.4±5.8 years), and nine cases of aged controls (average age: 74.8±13.9 years). The MS material comprised six patients with acute MS, seven patients with relapsing-remitting MS (RRMS), 18 patients with second-
ary progressive MS (SPMS), and 11 patients with primary progressive MS (PPMS); additionally, three patients with progressive MS were included, which could not be classified unequivocally as primary or secondary, and control cases were without neurological disease and neuropathological lesions. Clinical histories were available for all of the cases, and diagnosis of MS was histologically confirmed by a neuropathologist. Neuropathological analysis and immunohistochemistry were performed on sections from different brain areas.

For basic classification of inflammation, demyelination and diffuse white matter injury sections were stained with H&E, Luxol fast blue myelin stain, and Bielschowsky silver impregnation. Demyelinating activity of the lesions was defined by the presence of myelin degradation products within macrophages as described earlier [16].

Immunolabeling for HMGB1 was performed on 5 μm tissue cryosections using an avidin-biotin-HRP complex procedure as described previously. Briefly, blocking serum (10% human serum, Sigma Chemical Co., St. Louis, MO, USA) was applied at room temperature to the sections for 2 h prior to incubation with the primary polyclonal rabbit anti-HMGB1 antibody (Phar-Mingen, San Diego, CA, USA) or the astrocyte marker α-4-glial fibrillary acidic protein (GFAP; Neomarkers, Fremont, CA, USA) for 20 h at 4°C. Secondary antibody, goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA), was applied at room temperature for 1 h followed by incubation with peroxidase-labeled avidin (Sigma Chemical Co.). Primary and secondary antibodies were diluted in 10% human serum. The peroxidase reaction was visualized with diaminobenzidine (Fluka, Switzerland).

**Patients and controls used for real-time quantitative RT-PCR**

The MS cohort (collected at the Neurology Clinic, Karolinska University Hospital, Sweden) included 34 subjects (mean age, 44 years; range, 22–65 years; 24 females and 10 males; 25 with and four without IgG oligoclonal bands in CSF), fulfilling the McDonald criteria of MS [17]. Of these, 22 patients displayed RRMS and 12 SPMS disease courses. In the RRMS group, 10 CSF samples had been collected during relapse and 12 during remission.

The noninflammatory control (NIC) group (n=30) comprised patients with other noninflammatory neurological diseases (n=20; unspecified sensory disturbance, n=5; migraine, n=3; tension headache, n=12) and healthy individuals (n=10), as determined using a variety of neurological and neuroradiological examinations (mean age, 33.5 years; range, 15–62 years; 27 females and 3 males; all without IgG oligoclonal bands in CSF). Paired CSF and blood samples were available from all MS patients and NIC. The local ethical committee at Karolinska University Hospital approved the study.

**Preparation of PBMC and CSF-mononuclear cells (MC)**

Peripheral blood was sampled in sodium citrate-containing cell preparation tubes (Vacutainer CPT, BD Biosciences, San Jose, CA, USA) and CSF in siliconized glass tubes. CSF samples were centrifuged immediately, and the pellet and supernatant were recovered and stored at −70°C until use. PBMC were separated by density gradient centrifugation. Cells from the interphase were collected and washed twice in Dulbecco’s PBS. The proportion of viable cells was assessed by trypan blue exclusion, and more than 95% were viable. The cells were finally pelleted, frozen on dry ice, and stored at −70°C until use.

**Relative quantification of mRNA by real-time quantitative RT-PCR**

Cell pellets were lysed, and total RNA was extracted (Qiagen total RNA extraction kit, Hilden, Germany). Samples were incubated with 27 μK dNase (Qiagen; RNase-free dNase set) for 30 min at room temperature to avoid amplification/detection of contaminating genomic DNA. RT was performed using 10 μl total RNA, random hexamer primers (0.1 μg; Life Technologies, Paisley, Scotland), and Superscript RT (200U; Life Technologies). Quantitative analyses of mRNA expression were performed using QuantiTect™ SYBR® Green, according to the manufacturer’s instructions (Qiagen), and amplification was performed using an ABI PRISM 7700 sequence detection system (PerkinElmer, Norwalk, CT, USA). All primers were designed using the Primer Express Software (PerkinElmer) in our laboratory.

The sequences of all primers used in this study are as follows: GAPDH [18] HMGB1 forward primer, 5’-CTC AGA GAG CTG GAA GAC CAG CT-3’, reverse primer, 5’-GGG ATG TAG GTT TTC ATT TCT CTT GC-3’; RAGE forward primer, 5’-GAG GAG GAG CGT GCG CAA GT-3’, reverse primer, 5’-CTT CAA GCC CTT CCA GTA CTA GT-3’; TLR2 forward primer, 5’-TTC TCA TCT CAC AAA ATT GCA AAT-3’, reverse primer, 5’-GGA AGG TAA GTC CAG CAA AAT CCT-3’; and TLR4 forward primer, 5’-CTG CAA TGG ATC AAG CAC GAG-3’, reverse primer, 5’-TGC CCT GCT TAT CTG AAC GTG-3’.

**Western blot analysis**

Cell-free CSF (1.5 ml samples) was ultrafiltered using first 100 kD and then 10 kD Centrifion filtration devices (Millipore, Bedford, MA, USA) to a volume of 20 μl, boiled in SDS sample buffer containing protease inhibitors (Bio-Rad, Hercules, CA, USA), separated by SDS-PAGE, and transferred to nitrocel lulose membranes (Amersham Biosciences, Little Chalfont, UK). Blots were incubated with primary HMGB1 antibody (1:500, PharMingen) and detected using goat anti-rabbit HRP-conjugated secondary antibody (Amersham Biosciences) and a chemiluminescence kit (Amersham Biosciences).

**Spinal cord tissue from animals with EAE**

Female Dark Agouti (DA) rats were anesthetized with isoflurane (Abbott Laboratories, North Chicago, IL, USA) and immunized by s.c. injection in the dorsal tail base with 200 μl inoculum containing 20 pg recombinant rat myelin oligodendrocyte protein (MOG) in saline emulsified (1:1) with IFA (Difco, Detroit, MI, USA). Animals were weighed and examined daily by two alternating investigators for clinical signs of EAE as follows: Grade 1, Flaccid tail or tail paralysis; 2, hind limb paresis; 3, hind limb paralysis; 4, tetraparesis, moribund state, or death. Organs were dissected, embedded in paraffin wax, and sectioned at 5 μm. The local ethical committee approved this experimental study.

**Histopathology and immunohistochemistry of rat spinal cord**

Histopathological evaluations of paraffinembedded-hyde-fixed, paraffin-embedded sections of the spinal cord sampled on Days 0, 13, 19, or 29 postimmunization (p.i.) were made. The spinal cord was divided into multiple parts from cervical to sacral regions, and 17 pieces of different rostro-caudal levels of the spinal cord were embedded in paraffin. For basic classification of inflammation, serial deparaffinized sections were stained with H&E or Klüver-periodic acid-Schiff (PAS) myelin stain to assess inflammation and demyelination, respectively.

Similar sections processed for immunohistochemistry were pretreated using an antigen-retrieval technique consisting of 2 × 5 min boiling in 10 mM sodium-citrate buffer (pH 6.0) at 97°C, followed by blocking in 5% normal serum diluted in PBS0.1% Saponin. To detect the presence of specific cellular and extracellular markers in CNS macrophages and microglia, sections were incubated with a polyclonal rabbit anti-HMGB1 antibody (PharMingen), a mouse anti-TLR2 mAb (T2.5; Gene Tex Inc., San Antonio, TX, USA), polyclonal goat anti-RAGE antibody (Bio Logo, Krefl, Germany), mouse anti-TLR4 mAb (HTA 125; Abcam, Cambridge, UK), and/or mouse anti-ED1 mAb (Serotec, Oxford, UK). Incubation with primary antibodies was performed overnight at 4°C. The secondary antibodies applied were Alexa-donkey anti-goat, Alexa-donkey anti-rabbit, or Alexa-donkey anti-mouse antibodies (Molecular Probes, Holland).

**In situ hybridization**

Paraffin-embedded sections were mounted onto microslide slides (SuperFrost Plus, Pittsburgh, PA, USA) and pretreated as for immunohistochemistry. A synthetic oligonucleotide (Cyberyne AB, Huddinge, Sweden) complementary to rat HMGB1 mRNA, as described previously [19], was labeled with 35S-dATP (DuPont NEN, Boston, MA, USA) at the 3’-end using terminal deoxynucleotidyltransferase (Amersham Biosciences) and purified through QIAquick spin columns (Qiagen). Sections were hybridized at 42°C with 0.5 mg/labeled probe (1×10⁶ cpm/mg oligonucleotide probe) per slide in a cocktail as described earlier [20]. Sections were washed for 1 h in 1× SSC buffer at 55°C and another hour at room temperature, followed by rinsing in distilled water and dipping sequentially in 60% and 90% ethanol. For control purposes, an excess (100%) of cold probe was added to the hybridization cocktail. After air-drying, the sections were exposed to 13C-standard to Kodak
Biomax MR film (Kodak, Rochester, NY, USA). The films were developed for 5 min in Kodak LX 24 and fixed in Kodak AL4, rinsed in running tap water for 30 min, and air-dried.

Culture and activation of rat bone marrow-derived macrophages and primary microglia

Bone marrow-derived macrophages were prepared as described previously [21]. Primary microglial cells were isolated from DA pups (maximum 2 days old) using a modified protocol described previously [22]. Briefly, brains were mechanically dissociated before enzymatic dissociation with trypsin-EDTA (Life Technologies). Cells were then seeded into poly-L-lysine (10 μg/ml)-coated flasks and cultured in complete DMEM containing 20% FBS. After 24 h, the medium was replaced, and half of the medium was changed every 2nd day. After 10 days, the cultured microglia were shaken manually from the astrocyte layer. Cells were seeded into 24-well plates at a density of 2 × 10^5 cells per well in complete DMEM and rested overnight. Cells were then stimulated by addition of LPS (100 ng/ml; Difco) and recombinant IFN-γ (100 U/ml; kind gift from Dr. Peter van der Meide, Utrecht University, The Netherlands) for 20 h, fixed, and stained as described above.

Antibody specificity tests

Controls of specificity of staining procedures were based on parallel staining studies with the secondary antibodies alone and by using an irrelevant primary antibody in each assay.

Statistical analysis

Differences in quantitative HMGB1 levels in MS lesions and control CNS and relative mRNA levels of HMGB1, RAGE, TLR2, and TLR4 in CSF-MC and PBMC were tested for significance using the nonparametric Mann-Whitney test (GraphPad Prism 3.0, GraphPad, San Diego, CA, USA). A correlation between HMGB1 and receptor mRNA levels was analyzed using the Spearman’s rank test (GraphPad Prism 3.0).

RESULTS

High numbers of HMGB1-expressing macrophages and microglia are present in active MS lesions

To determine HMGB1 distribution in MS lesions, active and inactive lesions from MS autopsy brains were immunohistochemically stained. In actively demyelinating MS lesions, we recorded greater numbers of macrophages/microglia with cytoplasmic HMGB1 compared with controls (Fig. 1, A and C). (Upper graph) Active MS lesions (n=14) contain a significantly higher number of macrophages/microglia with cytoplasmic HMGB1 compared with controls (Ctrl.; n=16; P<0.0001). (Lower graph) Highest numbers of macrophages/microglia with cytoplasmic HMGB1 occur in sections from acute and RRMS, and the values from SPMS and PPMS are comparable with those from inactive lesions. The P values were calculated using the Mann-Whitney test; the horizontal bars represent median values.
Elevated HMGB1, RAGE, TLR2, and TLR4 expression in CSF cells from MS patients

As HMGB1 translocation was evident in active lesions from MS patients, we analyzed the relative expression of HMGB1 and its binding receptors RAGE, TLR2, and TLR4 in PBMC and CSF-MC of patients with MS and NIC. Using real-time RT-PCR, we first examined the expression of target mRNA in the PBMC of 34 patients with MS and 30 NIC. Although HMGB1 and receptor mRNA expression was detected in all PBMC samples, there was no significant difference in the relative expression of HMGB1 and TLR4 in the MS group as compared with the NIC group (Fig. 2A, upper). In CSF-MC, increased expression of all of the investigated molecules was significant in MS patients compared with in NIC (P<0.0001; TLR4, P<0.0005; Fig. 2A, lower). Furthermore, when mRNA expression of HMGB1 was compared with receptor mRNA expression in CSF-MC from the whole cohort, we determined a significant correlation between HMGB1 and each of the investigated receptors RAGE, TLR2, and TLR4 in CSF-MC (Sr, Spearman correlation). In accordance with these findings, Western blot analyses of identically concentrated CSF samples from MS patients and NIC revealed that MS patients had increased levels of soluble HMGB1 when compared with controls (Fig. 2C). Although this sample size studied is small, when sensitive, quantifiable immunoassays are available, it will be interesting to extend this study with a larger patient sample.
material, including kinetic analyses of CSF samples from individual patients with defined disease courses.

**HMGB1 expression follows clinical and histopathological disease in experimentally induced neuroinflammation**

We next examined the expression of HMGB1 in EAE, an immune-mediated animal model of MS [23]. Clinical signs of disease became evident from Days 9 to 13 p.i. and were corroborated by histopathological demonstration of active demyelination, signs of acute axonal injury, and dense infiltration of peripheral blood-derived monocytes into the CNS from Day 13 p.i. At disease onset, histopathology revealed diffuse infiltration of leukocytes into the CNS (Fig. 3A), and focal infiltration of immune cells with plaque formation was evident regularly during later relapse stages (Fig. 3C).

To monitor HMGB1 mRNA synthesis during EAE, in situ hybridization was performed on consecutive sections to those used for the immunohistochemical analyses. HMGB1 mRNA expression (Fig. 3D) in the lesions closely mirrored the number of infiltrating cells, signs of histopathological damage, and clinical symptoms (Fig. 3, A–C). ED1 immunohistochemical labeling was used as a marker of phagocytic cells (microglia/monocyte-derived macrophages), and the distribution of labeled cells had a large degree of overlap with H&E-stained, inflammatory cell infiltrates (Fig. 3, A–C). The observed hybridization signals temporally reflected the clinical score, with evident distinction between score 3⁻ (Day 13 p.i.; Fig. 3D, arrow A) and score 3⁺ (Day 29 p.i.; Fig. 3D, arrow B). On Day 29, there was an intense HMGB1 signal recorded, not only localized to the multiple lesions but also evident throughout the spinal cord section, indicating a widespread and significant inflammatory response in animals with severe EAE. Original scale bars, 500 μm (low magnification); 50 μm (high magnification); 3 mm (in situ).
spinal cord section, indicating a widespread and significant inflammatory response in animals with severe EAE (score 3+; Fig. 3D, arrow C). These data suggest that HMGB1 is synthesized de novo within EAE lesions.

**HMGB1 and the receptors RAGE, TLR2, and TLR4 are up-regulated in active lesions of MOG-EAE**

Based on the findings that HMGB1 and its receptors were up-regulated in MS patients and that HMGB1 mRNA expression was increased in CNS sections from MOG-EAE animals, we next sought to investigate the expression and localization of HMGB1 and its receptors RAGE, TLR2, and TLR4 in MOG-EAE lesions. Immunohistochemical staining revealed that in normal rat CNS tissue, HMGB1 was primarily located in the nucleus of most cells. In contrast, inflammatory CNS lesions of rats with EAE exhibited a distinctly different staining pattern, where nuclear HMGB1 expression was accompanied by a cytoplasmic staining in most ED1+ cells (green), ×100 original magnification. The receptors (B) RAGE, (C) TLR2, and (D) TLR4 are up-regulated within EAE lesions. Nonstimulated (E, inset) macrophages and (F, inset) microglia from DA rats display a preferential staining of HMGB1 (red) in the nucleus, as indicated by colocalization with 4',6-diamidino-2-phenylindole (DAPI; blue). After 24 h stimulation with LPS/IFN-γ, there is a significant accumulation of HMGB1 in the cytoplasm of (E) macrophages and (F) microglia. Original scale bars, 20 μm (A–D); 25 μm (E and F).

**DISCUSSION**

In this study, we demonstrate that HMGB1 and its receptors RAGE, TLR2, and TLR4 are highly expressed in active lesions of MS as well as in its counterpart animal model EAE, while being expressed at normal levels in inactive lesions. This suggests a potential interaction of these molecules in the inflammatory process involved in pathogenesis.

Interaction of HMGB1 with RAGE not only induces secretion of proinflammatory cytokines but also mediates up-regulation of cell adhesion molecule expression (i.e., ICAM-1, VCAM-1, and E-selectin) [24]. It has also been demonstrated that RAGE itself can lead to leukocyte recruitment through direct interaction with the β2-integrin membrane-activated complex 1 [25]. This could enable immunocompetent cells such as macrophages with increased RAGE expression to interact with the endothelium and transmigrate through the blood brain barrier into the CNS during MS and EAE. However, HMGB1/RAGE interaction may also exert a beneficial role during limited inflammatory responses, when HMGB1 and other ligands are present only briefly. In the setting of peripheral nerve injury, such as in the sciatic nerve crush model, blocking the ligand-RAGE interaction actually delays tissue repair [26]. Our findings therefore suggest that HMGB1 is a useful biomarker of ongoing neuroinflammation, although it may be playing inflammatory and/or anti-inflammatory roles.

The innate immune system exerts a central role in the initiation and regulation of subsequent antigen-specific immunity [27]. Recent data demonstrate that the innate immune system also plays an important role in the initiation of systemic autoimmunity [28, 29]. Pattern recognition receptors such as TLRs are important mediators of innate immune reactions, and TLR2 and TLR4 have additional roles as receptors for HMGB1. Expression of TLR2,
repair. Neural stem cells (NSC) have been demonstrated to mi-
trole of HMGB1 in stem cell chemotaxis and subsequent tissue 
recruitment into tissues [37]. These findings indicate a potential 
HMGB1 injection can induce blood vessel-associated stem cell 
remyelination, as well as decreasing neurite outgrowth. Notably, 
myelin debris clearance at the site of injury and subsequent 
Blocking the HMGB1–RAGE interaction in the damaged nerve 
context, nerve injury is associated with a transient and rapid rise 
ing on its post-translational modifications or that different splice 
it is possible that the biological activities of HMGB1 vary depend-
larly distinct from HMGB1 actively secreted by macrophages [35], 
findings, and HMGB1 may thus be an important biomarker de-
numer de novo synthesis of new protein, presumably to compensate 
HMGB1 in situ hybridization data from active EAE lesions sug-
gest de novo synthesis of new protein, presumably to compensate for 
active depletion through release, although this cannot be 
proven. However, this notion is supported by the immunohisto-
chemical stainings of MS and EAE lesions, revealing a cytoplas-
mic HMGB1 localization in macrophages and microglia (Fig. 4, E 
and F), although translocation from nucleus to cytoplasm does not 
necessarily lead to cellular release. To prove the exact relation-
ship among HMGB1 mRNA expression, cytoplasmic transloca-
tion, and extracellular release, it would be necessary to charac-
terize these for every cell type within the inflammatory lesion, and 
as yet, we lack assays sensitive enough to convincingly quantify 
extracellular release. In a model of cerebral ischemic injury, it was 
determined that HMGB1 released during the acute phase lacked 
hyperacetylation, indicating a necrotic origin of the protein, and 
HMGB1 levels continued to increase later during the chronic 
phase, suggesting active release from inflammatory cells. As MS 
and EAE are considered to be chronic diseases in which tissue 
damage is secondary to inflammation, this is consistent with our 
findings, and HMGB1 may thus be an important biomarker de-
fining disease chronicity.

As HMGB1 released passively from necrotic cells is molecu-
larly distinct from HMGB1 actively secreted by macrophages [35], 
it is possible that the biological activities of HMGB1 vary depend-
ing on its post-translational modifications or that different splice 
variants of RNA/protein isoforms have different activities. In this 
context, nerve injury is associated with a transient and rapid rise of 
HMGB1 and RAGE expression at the site of damage [36]. 
Blocking the HMGB1–RAGE interaction in the damaged nerve 
appears to down-regulate inflammatory mechanisms necessary for 
myelin debris clearance at the site of injury and subsequent 
remyelination, as well as decreasing neurite outgrowth. Notably, 
HMGB1 injection can induce blood vessel-associated stem cell 
recruitment into tissues [37]. These findings indicate a potential role of 
HMGB1 in stem cell chemotaxis and subsequent tissue repair. 
Neural stem cells (NSC) have been demonstrated to migr-
ate to the sites of lesions in EAE, differentiating into oligoden-
drocytes that would have an important role in remyelination [38]. 

However, in the same study, it was suggested that the migrating 
NSC were killed by activated macrophages. As HMGB1 thus has 
a putative role in NSC migration and differentiation as well as a 
known role in the activation of macrophages and microglia, which 
could lead to NSC death, it is possible that the balance of these 
different HMGB1/receptor interactions or alternatively, the cumu-
lative effects of HMGB1 and other immunological factors will 
decide the outcome of the local inflammatory reaction in terms of 
tissue damage or destruction.

Our present study also demonstrates that activation of micro-
glia leads to HMGB1 cytoplasmic translocation. Kim et al. 
[10] have recently described that microglia also respond in 
vitro to HMGB1 stimulation. Together, these findings indicate 
that macrophages and microglia have the ability to form a 
positive activation loop, and as these cells are able to release 
and respond to HMGB1, prolonged inflammation might be 
sustained. It is plausible that microglial production of HMGB1 
is regulated differently in vivo so that a chronic inflammation is 
prevented. Whether such specific regulation occurs and is 
inefficient in MS patients warrants further study.

Different strategies for inhibiting HMGB1 have been con-
ducted in various inflammatory animal models with ameliora-
tive effects: Soluble (s)RAGE, anti-HMGB1- and anti-B-box 
antibodies, and A-Box antagonists having been reported to 
prevent endotoxemia, sepsis, arthritis, acute lung injury, and 
hepatic injury, and sRAGE also prevents EAE [39]. As 
HMGB1 also has a defined role in neurite outgrowth [40] and 
stem cell chemotaxis [38], inhibition of HMGB1 at the wrong 
time might block tissue repair processes rather than quench 
inflammation, and thus, there is an inherent risk in using 
anti-HMGB1 treatment in the CNS.

In conclusion, we propose that HMGB1 in the CNS is a 
useful biomarker and may contribute to the chronicity of neu-
roinflammation through the proposed positive feedback loop 
involving infiltrating macrophages and resident microglia. We 
suspect that a finely tuned balance of HMGB1 and its receptors 
are at play to control this positive feedback system. We suggest 
that the balance of the involved molecules is crucial and that 
the restoration of this balance can enable a beneficial, inflam-
matory response and sustain the repair capabilities of HMGB1, 
which may be essential in MS remission.

**ACKNOWLEDGMENTS**

This work was supported by grants from Karolinska Institutet 
Fonder, the Swedish Association of Neurologically Disabled, 
and the Swedish Research Council. The authors thank Prof. M. 
Wahren-Herlenius and Dr. H. Erlandsson-Harris for critical 
reading of the manuscript.

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