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REVIEW

When the Brain Signals Danger: Roles of Calreticulin and HMGB1 in Cell Death Mechanisms in Neurooncology and Neurodegeneration

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Abstract—Contrary to the classical paradigm of brain immune privilege, damage-associated molecular patterns (DAMPs) act as key mediators in the dialogue between CNS cells and the immune system, determining pathways of immune response development. This review examines the role of key DAMPs – calreticulin (CRT) and high-mobility group box 1 protein (HMGB1) – in the functioning of CNS under normal conditions and during the development of neuropathologies, particularly oncological and neurodegenerative processes. Under normal conditions, CRT and HMGB1 ensure functioning of the brain, acting as necessary effectors of neurogenesis and homeostatic plasticity. In neurooncology, exposure of CRT on the surface of dying cells and release of HMGB1 serve as powerful adjuvant signals for antigen-presenting cells, inducing immunogenic cell death (ICD). Particular attention is given to the mechanisms of passive HMGB1 release during various types of regulated cell death (apoptosis, necroptosis, pyroptosis, ferroptosis, cuproptosis), as well as to the role of redox modifications of the protein, which determine its pro-inflammatory, immunogenic, or tolerogenic properties. In neurodegeneration (Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis), excessive DAMP release exacerbates the development of chronic neuroinflammation, disrupts integrity of the blood-brain barrier (BBB), and forms a vicious cycle of pathological changes. Detection of CRT and HMGB1 in biological fluids or brain tissues has important prognostic potential, and regulation of their signaling pathways opens promising approaches for overcoming tumor resistance or inhibiting progressive neurodegenerative changes in the brain.

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INTRODUCTION

For a long time, immunology was dominated by the idea that activation of the immune response occurs according to the "self vs non-self" principle. In the late 19th century, I. I. Mechnikov discovered the process of phagocytosis of foreign material and described one of the main mechanisms of innate immunity. In 1989, Charles Janeway proposed the concept of "pattern recognition" (Fig. 1), describing specific recognition of the conserved pathogen molecules, called pathogen-associated molecular

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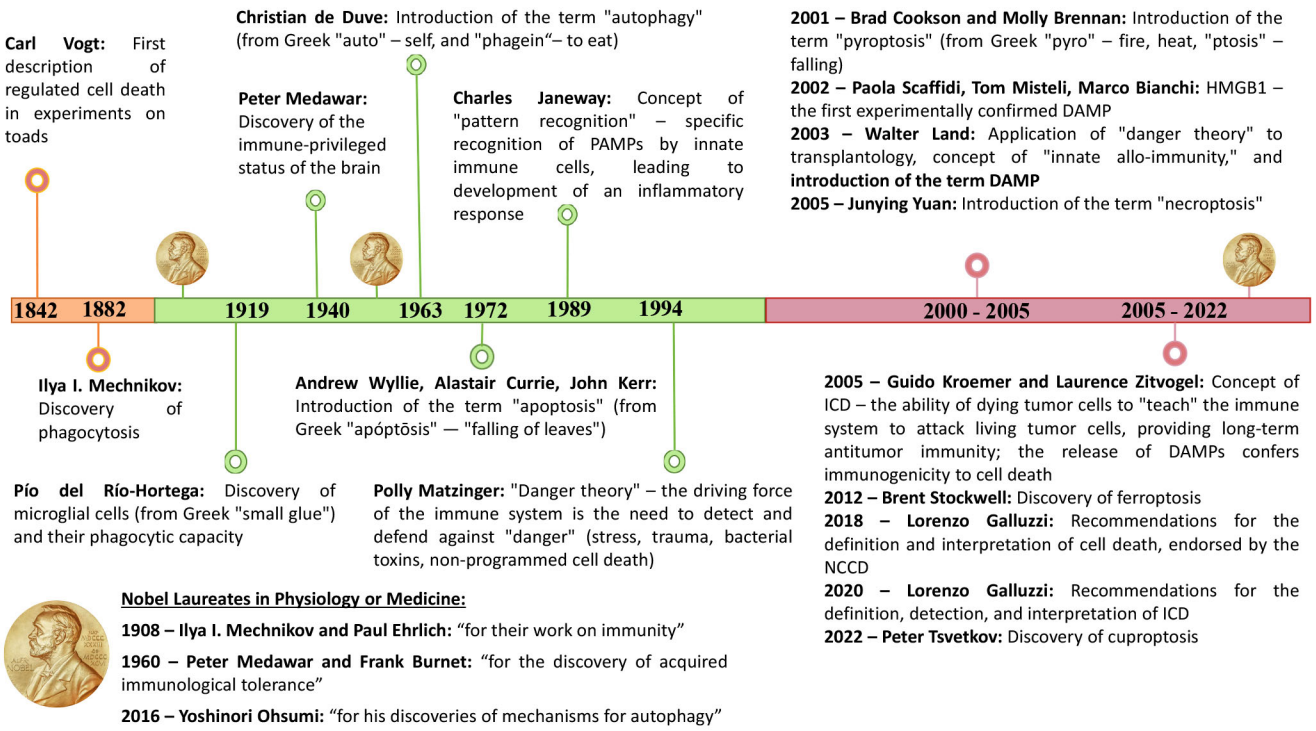

Fig. 1. From PAMPs and DAMPs to regulation of immune response and cell death: brief chronology of key discoveries.

patterns (PAMPs) [1]. PAMPs are recognized by the pattern-recognition receptors (PRRs), are encoded in the germline and expressed by the innate immune cells, which triggers an inflammatory response and protects the host from pathogen invasion [1]. Later, other factors leading to initiation of the immune response were discovered, mediating development of the so-called sterile (i.e., non-infectious) inflammation. In 1994, Polly Matzinger (Fig. 1) proposed the "danger theory", according to which the key task for the immune system is to identify and neutralize "danger", which could be bacterial toxins, viruses, tumor processes, or unprogrammed cell death [2].

Later, the author suggested a more generalized term for the immune response inducers as "danger signals from the damaged or stressed cells" [3], and in 2003, Walter Land expanded this concept to the term "damage-associated molecular patterns" (DAMPs) [4].

Unlike PAMPs, DAMPs comprise a heterogeneous group of endogenous molecules, including proteins, lipids, nucleic acids, which ensure normal functioning of the cell. In endogenous and exogenous damage, DAMPs could function as danger signal transmitters through open hydrophobic fractions and regulate both innate and adaptive immune responses [5]. If the level of stress exceeds the cell's repair capacity, regulated cell death is triggered (Table 1). The nature of this death may be silent, tolerogenic, inflammatory, or immunogenic, depending on the mechanism of cell death, the presence of antigens and adjuvants, and the characteristics of the microenvironment [6].

In infection, stress, or cell death, DAMPs are relocated to the extracellular space, where they could interact with PRRs, such as Toll-like receptors (TLRs), NOD-like receptors (NLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), C-type lectin receptors (CLRs), and others [7]. DAMPs could also be recognized by the receptor for advanced glycation end products (RAGE), triggering receptors expressed on the myeloid cells (TREMs), and by the G-protein-coupled receptors (GPCRs) [8-10]. Depending on the initiating stress, which determines the mechanism of cell death, the dynamics of DAMP release, their combinations with other molecules, and microenvironment, all these factors could have a dual role in the development of the immune response.

On the one hand, DAMPs could act as adjuvants for antigen-presenting cells, promoting activation of their phagocytic activity and maturation, as well as subsequent presentation of antigens by the major histocompatibility complex class I (MHC I) molecules to the $CD8^+$ T cells, leading to formation of an adaptive immune response and long-term immunological memory. This mechanism is called immunogenic cell death (ICD) (Table 1) [13]. To date, immunogenic properties have been shown for apoptosis, as well as other types of regulated cell death, including necroptosis, ferroptosis, pyroptosis, and the recently discovered cuproptosis (Table 1). Induction of ICD is considered

Table 1. Types of regulated cell death with immunogenic properties: a brief glossary

Name	Characteristics	References
Regulated cell death	a form of cell death initiated by activation of one or several signaling pathways, which could be partially or completely inhibited/enhanced as a result of genetic or pharmacological interventions, both under normal conditions and during the development of pathologies	[11]
Programmed cell death	a form of regulated cell death activated only under natural physiological scenarios as part of a genetically encoded program for the development of homeostasis in an organism	[11]
Accidental cell death (accidental necrosis)	an unregulated type of cell death caused by extreme physicochemical stress (e.g., thermal shock, severe freeze-thaw cycles) or mechanical damage; accompanied by the release of DAMPs into the extracellular space but lacks immunogenic properties	[12]
Immunogenic cell death (ICD)	a special form of regulated cell death activated in response to specific stimuli – ICD inducers (e.g., a number of chemotherapeutic drugs, radio-, photodynamic, and photothermal therapy regimens, viral load). The key feature of ICD is gradual release of DAMPs into the extracellular space, which, together with a number of chemokines and cytokines, act as adjuvants for antigen-presenting cells (e.g., dendritic cells). Contribution to immunogenicity is also made by the presence of antigens and neoantigens expressed by the dying cells. Close interaction between the adjuvant and antigen properties of ICD culminates in the presentation of antigens on major histocompatibility complex class I (MHC I) molecules to the $CD8^+$ T cells. This process ultimately leads to formation of an immune response and long-term immunological memory. Since the ICD mechanism is characterized not only by the release of DAMP patterns, the type of induced cell death and immunogenic properties must be confirmed not only *in vitro* but also in a series of experiments *in vivo* using syngeneic immunocompetent animals	[13-15]
Types of regulated cell death related to ICD		
Immunogenic apoptosis	an immunogenic apoptosis is characterized by the biochemical mechanism, for activation of which, in addition to caspase activation, ER stress processes and generation of reactive oxygen species are required. The mechanism is selectively blocked by the pan-caspase inhibitor zVAD-fmk (carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]-fluoromethylketone)	[16, 17]
Necroptosis	a caspase-independent form of programmed necrosis with immunogenic properties; triggered by the suppression of caspase-8 activity, followed by the sequential activation of the receptor-interacting protein kinases RIPK1 and RIPK3, and then of the pseudokinase MLKL, which forms pores in the membrane. The mechanism is blocked using specific inhibitors targeting RIPK1 (e.g., Nec-1s), RIPK3 (e.g., GSK'872), or MLKL (e.g., necrosulfonamide)	[12, 18]
Pyroptosis	a regulated form of immunogenic cell death accompanied by inflammation. Triggered by the assembly of an inflammasome – an intracellular oligomeric protein complex (e.g., NLRP1, NLRP3, NLRP4 [NOD-like receptors], IRAF, or AIM2), which promotes activation of caspase-1, or caspases-11, -4, -5. Under the influence of these proteases, gasdermin D (GSDMD) is cleaved to form an N-terminal fragment, which embeds in the plasma membrane. Initially, the cell exhibits morphological signs of apoptosis, but after membrane damage, the necrosis-like changes occur, manifested primarily as a sharp increase in membrane permeabilization. Culmination of death is accompanied by the release of intracellular contents, including DAMPs, as well as pro-inflammatory cytokines (e.g., IL-1β and IL-18), provoking a pronounced inflammatory response	[19, 20]

Table 1 (*cont.*)

Name	Characteristics	References
Ferroptosis	an iron-dependent form of regulated cell death mediated by generation of reactive oxygen species and lipid peroxidation; triggered by oxidation of polyunsaturated phosphatidylethanolamines by 15-lipoxygenases against a background of depleted intracellular glutathione and loss of function of the antioxidant enzyme GPX4. A decisive factor for ferroptosis is a shift in the redox balance towards pro-oxidants. The mechanism is blocked by the inhibitors such as ferrostatin-1 (Fer-1), liproxstatin, vitamin E (α-tocopherol), and coenzyme Q10	[19, 21]
Cuproptosis	a recently discovered type of regulated cell death mediated by accumulation of divalent copper (Cu^{2+}) in mitochondria. Copper, transformed by ferredoxin (FDX1) into a monovalent state, directly attacks lipoylated enzymes of the Krebs cycle (DLAT or DLST) or destroys iron-sulfur proteins. Culmination of death is accompanied by the development of proteolytic stress and oxidative damage to the cell. The mechanism is not blocked by the inhibitors of other types of regulated cell death, such as zVAD-fmk, necrostatin-1, ferrostatin-1, or by the oxidative stress suppressor N-acetylcysteine. Cuproptosis has not yet been included in the ICD concept, but data are emerging on its ability to transform the tumor microenvironment into an immunologically favorable state, contributing to the development of an adaptive immune response	[22, 23]

a promising strategy for anti-tumor therapy to overcome resistance and prevent cancer recurrence, as well as for treatment of other pathologies.

Development of sterile inflammation due to the DAMP-mediated activation of the innate immune system is necessary for tissue regeneration and recovery. However, it could become excessive and could transition into chronic inflammation, which is responsible for pathogenesis of many diseases, including auto-inflammatory diseases, metabolic syndrome, cardiovascular diseases, neurodegenerative diseases, and oncological diseases. Release of some DAMPs during the cell death could have an immunosuppressive effect [24]. Emergence of post-translational modifications of DAMPs could also modulate their functions in the immune system and provoke the pathology development [25].

Thus, deep understanding of whether the immune system recognizes DAMPs as a pathogenic signal or as a physiological regulatory stimulus is necessary for understanding homeostatic plasticity, causes of various pathologies, and development of effective treatment strategies. Such studies are particularly important in relation to the brain as an immune-privileged organ.

This review focuses on key representatives of DAMPs – calreticulin (CRT) and high-mobility group box 1 protein (HMGB1), most often considered in the context of immunogenic cell death and critically evaluates their role in the functioning of central nervous system (CNS) under normal conditions and during the development of neuropathologies, particularly oncological and neurodegenerative processes.

CALRETICULIN

General characteristics. History of the calreticulin (CRT) discovery continues to be a subject of active scientific discussion. The first mention of CRT was previously attributed to MacLennan's laboratory, which in 1974 described a high-affinity calcium-binding protein (HACBP) in a highly purified preparation of sarcoplasmic reticulum from the rabbit skeletal muscle [26]. In 1984, Waisman and colleagues isolated a new calcium-binding protein from the bovine liver cells and named it calregulin [27], which was later characterized in detail (1985) and partially sequenced (1987) [28]. In 1989, based on the nucleotide sequence of mouse cDNA, Koch's group determined amino acid sequence of the calcium-binding protein of the endoplasmic reticulum (ER) lumen, CRP55, and proposed the name calreticulin for it [29]. Later, it turned out that this protein is identical to calregulin, and the term calreticulin became widely accepted. After 50 years, the version of CRT discovery in 1974 as the HACBP protein was called into question, giving priority to calregulin. In a study conducted by Waisman's laboratory in 2024, evidence was provided that HACBP and calregulin are distinct proteins [28]. The analysis showed that HACBP is a complex of several muscle proteins,

and its existence as a separate molecule was not confirmed [30].

CRT is a highly conserved calcium-binding protein with molecular weight of about 46 kDa. CRT consists of 417 amino acids and three domains:

- a globular N-terminal domain, which mediates interaction with α-integrins and the DNA-binding site of the steroid receptor;
- a proline-rich P-domain, responsible for creating a lectin-like chaperone structure;
- a Ca^{2+}-binding C-domain, which mediates CRT interaction with other chaperones in the ER, followed by a KDEL motif at the carboxyl terminus, consisting of four amino acids (Lys-Asp-Glu-Leu), which ensures retention of the protein in the ER [30].

CRT is involved in more than forty intra- and extracellular processes in mammalian cells. The main site of CRT localization is ER, where it functions as a chaperone, in particular, ensuring quality control of glycoproteins, preventing export of the misfolded proteins, and supporting their refolding during ER stress [31]. In addition, CRT is involved in the regulation of calcium homeostasis and signaling [32], which is of particular importance for CNS cells, where calcium dynamics play a determining role in the synaptic transmission and plasticity [33].

On hippocampal neurons in rats, extracellular CRT has been shown to co-localize with the NMDA receptor, which potentially plays a role in modulating Ca^{2+} influx into neurons [34]. CRT is characterized by differential expression depending on physiological and pathological states under consideration [35]. In the experimental *in vivo* models, it has been shown that the elevated CRT levels are observed in the developing brain and retina, while its deficiency leads to the neural tube closure defects in the embryonic period [36] and development of neurodegenerative processes in the postnatal period [37-39]. In recent *in vitro* and *in vivo* studies, specific regulation of CRT expression by the transcription factor ATF6β has been established. The ATF6β-CRT interaction increases survival of the hippocampal neurons under ER stress and development of the kainate excitotoxicity through regulation of the intracellular calcium homeostasis [40].

CRT is an important component of the peptide-loading complex (PLC) in the ER membrane, which ensures assembly of the MHC I heavy chains with β_2-microglobulin, transport of the antigen peptides from the cytosol into the ER lumen, and their loading into the antigen-binding groove of MHC I, followed by the export of assembled complexes via the anterograde pathway to the Golgi apparatus and then to the plasma membrane of the cell [41, 42]. As part of the PLC complex, CRT maintains a pool of immature MHC I heavy chains and promotes return of the suboptimally assembled or dissociated molecules from the post-ER compartments for re-initiation of assembly [43].

CRT has been also found in the nucleus and cytoplasm, which indicates multicompartmental nature of the protein and emphasizes the wide range of its functions. The nuclear CRT mediates export of a number of nuclear receptors, including the glucocorticoid receptor [44]. The cytoplasmic CRT promotes its activation by binding to the α-subunit of integrin, which leads to the short-term increase in cytosolic Ca^{2+} concentration due to operation of the voltage-dependent L-type calcium channels and release of Ca^{2+} from the intracellular calcium depots mediated by inositol trisphosphate (IP3) [30, 45], and also ensures connection with the extracellular matrix proteins and the process of cell adhesion [30, 46].

“Eat me” signal: role of calreticulin as a DAMP. With the discovery of the process of CRT exposure on the surface of the plasma membrane as an “eat me” signal for phagocytosing cells, the era of considering it as a DAMP and its role in ICD mechanisms began [17, 47, 48], which acquired special significance in the context of anti-tumor immunotherapy, particularly in the therapy of CNS tumors. Since CRT plays an important role in maintaining intracellular Ca^{2+} homeostasis, its role was primarily associated with immunogenic apoptosis processes (Table 1). However, today it is understood that the CRT exposure is one of the three key DAMPs (along with adenosine triphosphate [ATP] and HMGB1) considered in the context of immunogenicity of various types of regulated cell death (Table 1). In response to an ICD inducer, transport of CRT to the outer surface of the cytoplasmic membrane usually occurs at an early stage of cell death due to ER stress, even before the exposure of phosphatidylserine, which indicates independence of the process from the nuclear components, DNA damage, and transcriptional changes associated with the induction of cell death [49, 50]. CRT release is most often mediated by the depletion of Ca^{2+} stores in the ER. ER stress stimulates the kinase PERK, which, in turn, phosphorylates the translation initiation factor eIF2α. This is followed by activation of caspase-8 and caspase-8-induced proteolysis of the ER protein associated with the B-cell receptors 31 (BAP31), as well as conformational activation of the pro-apoptotic proteins Bax (Bcl-2-associated X-protein) and Bak (Bcl-2 homologous antagonist/killer) [50, 51]. As a result of these processes, CRT transport to the Golgi apparatus occurs, accompanied by the production of reactive oxygen species [50, 51]. Finally, release of the CRT-containing vesicles to the surface of the plasma membrane is carried out via the SNARE-dependent exocytosis (soluble N-ethylmaleimide-sensitive factor

attachment protein receptors) [50]. Blocking any of the steps of this complex mechanism with pharmacological agents (e.g., CRT-blocking antibodies) or genetic interventions (e.g., short interfering RNAs (siRNAs)) nullifies the effect of CRT, reduces immunogenicity of the cell death process, and, as a consequence, effectiveness of the immune response [47].

When CRT appears on the surface of the cytoplasmic membrane (ecto-CRT), it binds to the low-density lipoprotein receptor (LRP1, also known as CD91). This event, together with the secretion of pro-inflammatory cytokines (such as IL-6 and TNF-α) by the professional antigen-presenting cells, forms a powerful phagocytic signal that enhances uptake of antigens and neoantigens, ensures their subsequent presentation to the cytotoxic T cells, and, ultimately, leads to activation of the adaptive immune responses [13, 52]. CRT could also move not only to the surface of dying cells but also of the phagocytes themselves, where it could act as a phagocytic receptor together with LRP1 [53]. It is worth noting that the immunostimulatory effects of ecto-CRT are inhibited by co-expression of the integrin-associated protein CD47, which acts as a phagocytosis inhibition signal (“don’t eat me” signal) by binding to the signal regulatory protein SIRPα on the surface of the antigen-presenting cells [54, 55]. A delicate balance between the levels of ecto-CRT and CD47 correlates with the efficiency of binding of dying cells to phagocytes, and thus with the effectiveness of the development of the subsequent immune response and the conducted therapy [55, 56].

Calreticulin as a marker of immunotherapy efficacy in neurooncology. In carcinogenesis, the autonomous mechanisms of tumor cells responsible for regulating a balanced functional proteome through disruption of signaling pathways (e.g., the PERK-eIF2α pathway) could also contribute to the retention of CRT inside the cell [48], or they could secrete mutant forms of CRT [57], disrupting signaling between the dying tumor cells and the immune system cells, thus reducing effectiveness of ICD. In particular, mutation in the 9th exon of the *crt* gene leads to formation of a modified protein lacking the KDEL motif for retention in the ER. Appearance of the mutant ecto-CRT in a number of solid tumors treated with drugs that induce immunogenic apoptosis (e.g., mitoxantrone, oxaliplatin) or PD-1 blockade led to suppression of phagocytosis of the dying tumor cells by dendritic cells, thereby suppressing the anti-tumor immune response and effectiveness of immunotherapy [57]. An increased level of the immunoglobulin-binding protein (Bip) – a marker of activation of the unfolded protein response – limited the release of DAMP patterns, including CRT, from the glioma stem cells when using ionizing radiation regimens that induce immunogenic apoptosis [58]. In contrast, inhibiting Bip facilitated the ionizing radiation-induced ER stress, which led to generation of DAMP molecules through phosphorylation of PERK and IRE1-α, further maturation of dendritic cells, and activation of the effector T lymphocytes, as well as prevention of the tumor growth and reduction in the frequency of relapses in the mouse *in vivo* model of orthotopic glioblastoma [58].

Release of DAMPs, including CRT, during the ICD activation could also play a role in the degree of immunogenicity manifestation and effectiveness of immunotherapy. It was recently shown that the pharmacological blockade of CRT or ATP in the whole-cell lysates of the ferroptotic glioblastoma GL261 cells significantly reduced effectiveness of the prophylactic dendritic cell vaccines in the *in vivo* orthotopic tumor model [59]. Restoring CRT or ATP levels did not lead to the significant improvement in animal survival, but it delayed formation of neurological deficits. This suggests that although individual DAMPs could partially enhance activation of the immune system, they are insufficient on their own to fully reproduce complex immunogenic profile induced by the ferroptotic cell death [21, 59].

Thus, deeper understanding of the DAMP signaling pathways, in particular CRT, as well as the reasons for their modulation in carcinogenesis, would help to establish a productive dialogue between the induction of ICD and effectiveness of the ICD-based anti-cancer immunotherapy.

In addition, in some studies, detection of the ecto-CRT is considered as an independent prognostic marker and biomarker of therapy efficacy. For example, immunohistochemical analysis of the neuroblastoma tissue obtained from 68 patients showed that the elevated CRT levels positively correlate with the tumor differentiation, favorable therapy outcome, and increased survival of the patients at the late stages of the disease [60]. In the subsequent *in vitro* experimental study by the authors using three different human neuroblastoma cell lines (SH-SY5Y, SK-N-DZ, and stNB-V1), it was shown that CRT hyperexpression suppresses proliferation and enhances differentiation of the tumor cells by increasing expression of HIF-1α, followed by activation of the pro-angiogenic factor VEGF-A [61]. Blocking the VEGF-A signaling pathway significantly suppressed expression of the neuronal markers GAP43, NSE2, NFH, and TrkA, which indicates the key role of VEGF-A in the neuronal differentiation of neuroblastoma cells induced by CRT [61]. It was also shown that controlling the CRT levels could affect sensitivity of the human neuroblastoma cells to radiotherapy and stem cell properties, which suggests a favorable prognosis for the patient [62]. In another *in vitro* study using two human glioblastoma cell lines (U251MG and T98G) and a human

neuroglioma cell line (H4), it was shown that the elevated CRT levels enhance sensitivity of the cells to γ-irradiation. In the case of CRT hyperexpression, disruption of Ca^{2+} homeostasis leads to upregulation of the protein phosphatase 2Ac alpha (PP2Ac alpha), which suppresses the Akt signaling pathway, thereby modulating the radiation-induced apoptosis [63].

Thus, CRT is an important protein in the process of immunogenic death of tumor cells. Its translocation and exposure on the cell surface in response to ICD induction serves as a signal that attracts phagocytes, followed by the development of a persistent anti-tumor immune response. Understanding the mechanisms of ecto-CRT exposure regulation and its interaction with the immune cells is an important direction for the development of new anti-tumor therapy strategies aimed at enhancing immunogenicity of the tumors.

Role of calreticulin in neurodegeneration. In the context of neurodegeneration, particularly Alzheimer's disease (AD), the release of CRT could act as an alarmin – a danger signal factor released by the damaged neurons to attract and activate microglia, as an extracellular chaperone to prevent β-amyloid (Aβ) aggregation, and as a neuroprotector against the Aβ-induced neurotoxicity [64, 65]. Thus, CRT helps maintain Aβ in a soluble state or promotes its degradation [66]. In a recent *in vitro* study, it was shown that the increasing concentration of ecto-CRT to nanomolar concentrations promotes attraction of microglia, thus activating production of the cytokines TNF-α, IL-6, IL-1β, and the chemokine CCL2 (C-C motif ligand 2). In addition to the changes in the morphological and functional profile of microglia, ecto-CRT contributed to the blocking of Aβ fibrillization and modification of its oligomerization, preventing the Aβ-induced neuronal death in the primary cultures of cerebral cortex and cerebellum cells of the Wistar rats (P 3-5) [64]. Moreover, it has been shown that CRT could interact with the amyloid precursor protein (APP) at its γ-secretase cleavage site, as well as with the components of the γ-secretase complex, presenilin, and nicastrin. Blocking or reducing the CRT levels, as well as disrupting the CRT binding to APP, allows APP to directly interact with presenilin and other components of the γ-secretase complex, which leads to the enhanced proteolytic cleavage of APP, increased production and aggregation of Aβ, contributing to the development of amyloid pathology [67]. This is consistent with the existing data on the significantly lower levels of both CRT mRNA and protein in the serum of AD patients compared to the healthy individuals, with the degree of reduction correlating with progression of clinical manifestations of the disease [68]. The reduced CRT expression has also been observed in the brain cells of AD patients [37]. This suggests that the reduced CRT levels in serum could serve as a diagnostic biomarker for AD.

However, there are alternative data suggesting a negative role of CRT in the development of AD. Knockdown of the *crt-1* gene reduced locomotion and resulted in mobility impairments in the *Caenorhabditis elegans* (*C. elegans*) overexpressing the most toxic human β-amyloid peptide (Aβ1-42). At the cellular level, suppression of the *crt-1* gene prevented the Aβ1-42-induced increase in mitochondrial respiration and formation of the mitochondrial reactive oxygen species, as well as it reduced the number and size of the Aβ1-42 aggregates in the body wall muscle cells of *C. elegans* [69]. Blocking of CRT inhibited the loss of neurons caused by microglial activation in culture and *in vivo* [70].

CRT is a common link with pathogenesis of other neurodegenerative diseases, such as Parkinson's disease (PD). Inhibiting CRT increased the death of neurons in the SH-SY5Y cell line when PD was modelled using 6-hydroxydopamine (6-OHDA). *In vivo*, reduction in the CRT and ERp57 levels was demonstrated in the midbrain of rats with the 6-OHDA-induced parkinsonism [71]. Similar data were obtained in the study of the effects of mutation in the *crt-1* gene in *C. elegans*, which led to the enhanced loss of dopaminergic neurons induced by 6-OHDA [72].

In the context of PD, the data indicating that CRT regulates the nuclear-cytoplasmic translocation of α-synuclein in a calcium-dependent manner attract a great interest [73]. α-Synuclein plays a critical role in the onset and progression of PD, forming aggregates that are the main components of Lewy bodies, characteristic of PD and other neurodegenerative diseases classified as synucleinopathies. Therefore, disruption of the CRT-mediated regulation of localization and nuclear-cytoplasmic translocation of α-synuclein may be a significant element in the pathogenesis of PD.

These data suggest that the reduced levels of the components of the CRT/CNX glycoprotein quality control system may play a role in the neuronal damage in PD and other neurodegenerative diseases associated with dysfunction of the ubiquitin-proteasome system.

In a recent study, it was shown that the patients with secondary-progressive multiple sclerosis have a significant increase in the CRT levels in cerebrospinal fluid together with other DAMPs, such as annexin A1 (ANXA1) and HMGB1, indicating the possibility of ICD induction and initiation of an adaptive autoimmune response [74]. In the mouse model of amyotrophic lateral sclerosis (ALS) (SOD1(G93A) line), it was shown that the CRT levels are reduced two-fold in the damaged motor neurons. With hemizygous deletion of SOD1(G93A);crt(+/−), earlier manifestation of the muscle weakness and muscle denervation was observed compared to the SOD1(G93A) mice [39].

Thus, changes in the level of expression and amount of extracellular CRT could be considered as a potential biomarker and prognostic criterion for various brain pathologies. However, further research is needed to understand the exact role of CRT in the development of neurodegenerative processes and the possibility of its use as a therapeutic target, since the currently available data are ambiguous and partially contradictory.

HMGB1

General characteristics. High-mobility group box 1 protein (HMGB1, also known as HMG1 or amphoterin) belongs to the family of high-mobility group (HMG) proteins, along with HMGA and HMGN proteins. This family of highly conserved non-histone proteins was discovered in 1973 in the calf thymus chromatin [75, 76]. The human HMGB1 protein has 99% homology with the rodent protein. It consists of 215 amino acids, forming two structurally conserved DNA-binding domains (HMG-box A and HMG-box B) connected by a linker, a negatively charged C-terminal region (acidic tail) enriched with aspartic or glutamic acid residues, and a short N-terminal fragment [25]. Extracellular HMG-box B could exhibit pro-inflammatory properties through interaction with the receptor for advanced glycation end products (RAGE) and TLR4 [77]. On the other hand, HMG-box A could inhibit this pro-inflammatory action by competing with the full-length HMGB1 or HMG-box B for binding to the same receptors. The anti-inflammatory function of HMG-box A is further enhanced when fused with the C-terminal acidic tail [78].

HMGB1 is widely represented in the tissues and organs performing a wide spectrum of functions. HMGB1 is intensively synthesized during the CNS development in the embryonic period, especially in its early stages [79], and is closely associated with neurogenesis and axonal growth processes. At these stages of development, HMGB1 is predominantly localized in the cytoplasm, controlling neurite growth and neuron maturation [80]. The HMGB1-CXCL12/CXCR4 signaling pathway, parallel to the innate immune system, was discovered in the developing CNS of mice [81], suggesting that HMGB1 is a key mediator of neuroimmune interaction. These same authors showed that the cells of primary cultures of the cerebral cortex obtained from the mice with knockout of the *Hmgb1* gene did not restore their proliferative activity when the recombinant HMGB1 was added, which emphasizes the important role of the protein in regulating division of the neuronal precursors [81].

At the later stages of ontogenesis, the level of HMGB1 significantly decreases, and in the adult brain, it is mainly concentrated in the areas with preserved neurogenesis, such as dentate gyrus of hippocampus, olfactory bulb, and granular layer of the cerebellar cortex [79, 82]. In recent studies on the sexually mature CD-1 mice, it was shown that HMGB1 could cross the blood-brain barrier (BBB) in both directions – from the systemic circulation to the CNS and from the CNS back into the bloodstream, which potentially suggests its role in maintaining CNS homeostasis and regulating neuroinflammation [83]. Thus, the role of HMGB1 in the adult brain is not limited only to regulation of neurogenesis, and the spectrum of its functions in the CNS under normal conditions and during the development of pathologies requires further in-depth study.

Features of HMGB1 localization. Realization of the HMGB1 functions is largely determined by its location in a particular cellular compartment.

In the nucleus, HMGB1 ensures maintenance of the genome stability, acting as a non-histone chromatin-associated protein and DNA chaperone. In particular, HMGB1 actively participates in the regulation of transcription of hundreds of genes and several transcription factors and is also involved in the DNA repair systems and recombination processes [84].

When HMGB1 is translocated to the cytoplasm of the cell, it binds to Beclin-1 (BECN1), a central protein that initiates assembly of the Vps34/PI3K-III complex and formation of autophagosomes, which stimulates the process of autophagy [85]. Interaction of HMGB1 with BECN1 also leads to the disruption of its connection with the anti-apoptotic protein Bcl-2, which suppresses apoptosis. A special role of HMGB1 in regulating complex interaction between the autophagy and apoptosis has been shown in carcinogenesis. Increased levels of the cytoplasmic HMGB1 contribute to the development of resistance of tumor cells to therapy by enhancing autophagy as a survival mechanism [86, 87].

Regulation of the HMGB1 transport between the nucleus, cytoplasm, and extracellular space is influenced by a number of post-translational modifications. Particular importance is attached to the oxidative-reductive modifications of three cysteines (C23, C45, C106), the degree of oxidation of which, occurring due to the development of stress and generation of reactive oxygen species, regulates release of the protein into the cytoplasm [25, 88] and further into the extracellular space, where HMGB1 could both induce and suppress the immune response. The state of thiol (-SH) groups of HMGB1 is influenced by the balance of peroxiredoxins Prx1 and Prx2, which catalyze oxidation of HMGB1 [89], as well as of thioredoxin and glutathione pathway, which inhibit oxidation of HMGB1 [90].

The fully reduced HMGB1, fr-HMGB1, (all three residues in thiol form) is located in the nuclear space

and is necessary for structural and regulatory functions [88, 91]. When released into the extracellular space, fr-HMGB1 forms a complex with CXCL12 and acts as a chemoattractant for immune cells [92]. The partially oxidized HMGB1, ds-HMGB1, (disulfide C23-C45) has pronounced pro-inflammatory activity and is responsible for regulating autophagy [91]. The fully oxidized HMGB1, ox-HMGB1, is devoid of chemokine and cytokine functions [25, 93].

When released into the extracellular space, HMGB1 could act as a DAMP, stimulating reactions of the innate immune system. The extracellular HMGB1 could bind to several PRRs, including the specific receptor for advanced glycation end products (AGER, better known as RAGE), TLR2, and TLR4 [94, 95], which trigger the pro-inflammatory signaling pathways, cytokine and chemokine production, and recruitment of immune cells. This ability determines the central role of this protein in initiation of sterile inflammation and formation of immune response in various pathologies.

Active studying of the functions of extracellular HMGB1, including its role as a DAMP, began after the discovery in 1999 of extracellular secretion of HMGB1 by the lipopolysaccharide-induced macrophages, followed by the development of inflammation, which was experimentally confirmed in the *in vivo* model of lethal endotoxemic shock [96]. Later, it became known that HMGB1 could be secreted not only from the immune system cells, such as microglia, macrophages, neutrophils, T cells, but also from astrocytes and neurons [97]. In a recent study, the release of HMGB1 in extracellular vesicles by the cortical neurons in response to a single spreading depolarization, bypassing microglia, was established [98]. The vesicles are absorbed by the perisomatic processes of astrocytes through a narrow interstitium, initiating sterile cell-free neuroinflammation. Such neuron-astrocyte interaction ensures transmission of the narrowly directed physiological inflammatory signal to the meninges, informing about transient brain activity disorders, but without the subsequent development of the generalized inflammatory response (e.g., absence of microglial activation and degradation of tissue architecture), characteristic of classical inflammation [98].

Active and passive release of HMGB1 into extracellular space. Release of HMGB1 into the extracellular space occurs in two main ways: active secretion or passive release.

Unlike other soluble secreted proteins, absence of a leader sequence in HMGB1 does not allow it to be actively secreted via the traditional ER–Golgi secretory pathway [99]. To date, the most widely accepted model is that active release of HMGB1 occurs by packaging the protein into the intracellular vesicles (e.g., lysosomes) followed by their fusion with the plasma membrane and release into the extracellular space [25, 76]. The mechanism includes three main stages:

- acetylation of HMGB1 and its subsequent chromosomal dissociation;
- export of HMGB1 from the nucleus to the cytosol using the transport protein exportin 1 (XPO1, also known as CRM1);
- aggregation of HMGB1 in lysosomes and release outward through lysosomal exocytosis [76, 100].

A number of studies note that the mechanism of active HMGB1 secretion could be regulated by post-translational modifications (ADP-ribosylation, phosphorylation, methylation, acetylation, glycosylation), level of reactive oxygen and nitrogen species, ionic homeostasis (primarily Ca^{2+}), activation of transcription factors and inflammasomes, as well as by the features of intercellular interactions [25, 76]. Deep understanding of this regulatory network opens opportunities for the development of targeted therapy aimed at selective inhibition of the pathological release of HMGB1 without disruption of its physiological functions.

Passive release of HMGB1 is detected during the uncontrolled cell death (necrosis) and autophagy-mediated cell death, as well as at the late stages of the regulated cell death pathways, including apoptosis, necroptosis, pyroptosis, ferroptosis (Fig. 2).

HMGB1 released during necrotic cell death is predominantly in the disulfide or partially oxidized form; it enhances pro-inflammatory reactions through the TLR4 and RAGE signaling pathways. This is of particular importance as an early marker of inflammation in the CNS pathologies such as ischemic stroke, traumatic brain injuries, and active neurodegenerative processes, where massive cell death leads to rapid accumulation of HMGB1 and development of sterile neuroinflammation.

At the late stage of apoptosis (Table 1), passive release of HMGB1 from dying cells occurs in a caspase-3-dependent manner [101], followed by the development of ICD [94], or immunological tolerance in the case of presence of the oxidized form of the protein [102] (Fig. 2).

In the case of necroptosis (Table 1), development of the RIPK1-dependent inflammation occurs, mediated by the HMGB1-TLR4 signaling pathway. The TLR4 signaling pathway is involved in the secretion of HMGB1 from macrophages through the mechanism dependent on interleukin-1 receptor-associated kinase 4 (IRAK4) [18, 103]. The release of HMGB1, due to induction of the RIPK3-MLKL-dependent necroptosis in the hippocampus, contributes to the development of neuroinflammation and formation of the depression-like behavior in mice [104]. Inhibiting RIPK1

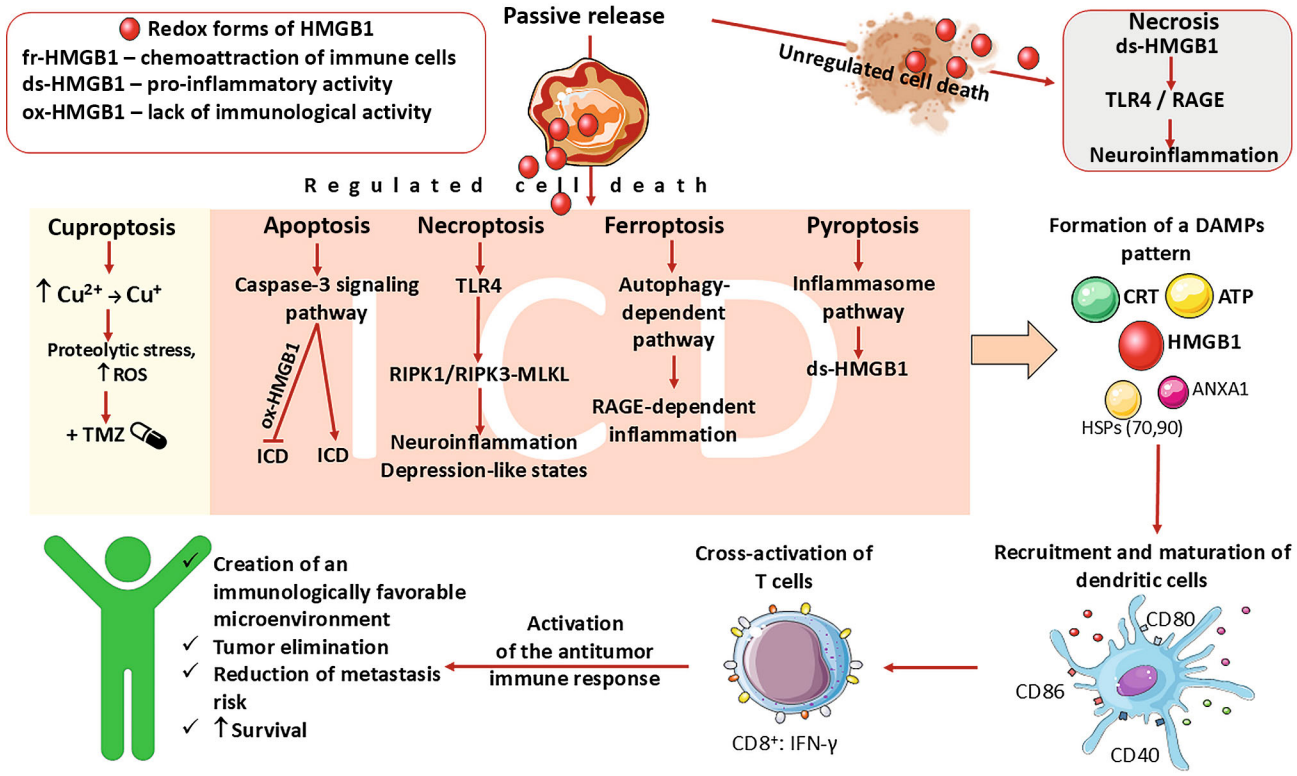

Fig. 2. Key mechanisms of passive HMGB1 release into the extracellular space. Passive release of HMGB1 occurs during uncontrolled cell death (necrosis), as well as at the late stages of regulated cell death pathways, including apoptosis, necroptosis, pyroptosis, ferroptosis, cuproptosis. Redox status of HMGB1 determines the pathway of immune response activation: development of sterile inflammation, induction of ICD, or immunological tolerance. In the case of ICD induction, HMGB1 released into the extracellular space, together with other danger molecules, forms a DAMP pattern that acts as a powerful adjuvant for the recruitment and maturation of the antigen-presenting cells (e.g., dendritic cells), followed by formation of an adaptive T-cell anti-tumor immune response.

and/or RIPK3, or depleting the pseudokinase MLKL, blocks the release of HMGB1 in various inflammatory conditions [105] (Fig. 2).

Unlike apoptosis and necroptosis, HMGB1 released during pyroptosis (Table 1) via the inflammasome pathway [106, 107] is in the hyperacetylated state and at the final stages of death is present in the disulfide form [108]. The JAK/STAT1 signaling pathway ensures hyperacetylation of HMGB1 and its cytoplasmic accumulation for its subsequent release into the extracellular space from the immune cells induced by lipopolysaccharide [109]. In a recent study, it was shown that the release of HMGB1 during inflammasome activation is GSDMD-dependent, but the release occurs not through the GSDMD pores, but due to membrane rupture [110]. Caspase 8 (Casp8) could also contribute to the GSDMD-dependent pyroptosis with HMGB1 release during the infection development [111] (Fig. 2).

Currently, it is known that the release of HMGB1 from ferroptotic cells (Table 1) occurs via an autophagy-dependent mechanism. It was shown as a result of testing of a wide range of ferroptosis inducers in both tumor and healthy cells that the autophagy-mediated inhibition of histone deacetylase (HDAC) leads to the HMGB1 acetylation, its release from the dying cells, followed by the development of the RAGE-dependent inflammation in macrophages [112] (Fig. 2).

Thus, passive release of HMGB1 into extracellular space could occur both via immunogenic and non-immunogenic pathways.

Role of HMGB1 in neurooncology. In the context of oncological diseases, particularly brain tumors, HMGB1 could play a dual role.

On the one hand, HMGB1 is involved in the tumor invasion and metastasis processes and is considered by a number of researchers as a therapeutic target. For example, the use of temozolomide increases the level of expression of HMGB1 in the glioblastoma cells obtained from the patient biopsies and promotes its further release into the tumor microenvironment. There, through activation of the TLR2/NEAT1/Wnt signaling pathway, HMGB1 enhances production of the cancer stem cells, ensuring development of resistance to the conducted therapy [113]. The authors consider combination of temozolomide with an HMGB1

inhibitor as a new strategy to overcome resistance and improve quality of the conducted therapy. In the animal models, blocking the HMGB1–RAGE interaction contributed to reduction in the growth and metastasis of both grafted and spontaneously developing tumors [37]. The use of neutralizing anti-HMGB1 antibodies contributed to the reduction in the vessel permeability and edema in the microenvironment of orthotopic glioma, increasing survival of the mice receiving oncolytic viral therapy [114].

The main mechanism by which the extracellular HMGB1 acts as a DAMP and is an important component of immunogenicity of the cell death lies in activation of the myeloid differentiation primary response protein (MYD88) of the TLR4 pathway and its adapter on the surface of antigen-presenting cells [94, 115]. This activation underlies processing and presentation of antigens from the dying cells, a necessary component of the further development of adaptive immune response. Desensitization of the tumor microenvironment to the TLR4 receptor or oxidation of HMGB1 under the action of ROS could lead to inactivation of TLR4, suppressing phagocytic activity of the antigen-presenting cells and development of immune tolerance [116]. It has also been shown that the release of HMGB1 from the dying glioma cells due to effects of the combined therapy with an adenoviral vector and radiochemotherapy based on temozolomide contributes to infiltration of dendritic cells and regression of the TLR-2-dependent brain tumors *in vivo* [117].

In recent studies, it has been established that the release of HMGB1 in combination with other key DAMPs during activation of cuproptosis (Table 1) in the glioblastoma cells demonstrates a synergistic anti-tumor effect in combination with temozolomide, as well as contributes to the development of an adaptive immune response, including ensuring maturation of the dendritic cells and remodeling of the tumor microenvironment, stimulating infiltration of the $CD8^+$ T cells and reducing intensity of the tumor growth [118, 119] (Fig. 2).

However, the release of HMGB1 should be attributed rather to the general signs of cell death (the fact of membrane permeabilization) than to the specific markers of ICD [13, 120]. For example, unlike CRT and ATP, pharmacological blockade of the HMGB1-TLR4 pathway in the whole-cell ferroptotic lysates of the glioblastoma GL261 cells did not have a significant effect on the effectiveness of prophylactic dendritic cell vaccines in the orthotopic tumor *in vivo* model [59]. However, blocking all three DAMPs in the ferroptotic lysates of the glioblastoma cells led to the maximum suppression of immunogenicity of the dendritic cell vaccines [59].

Thus, for complete characterization of immunogenicity of the cell death process, it is worth considering dynamics of the release of the entire DAMP pattern, including the release of CRT and ATP. According to the "gold standard" for defining ICD, it is also necessary to conduct studies to establish phenotypic status and phagocytic activity of the antigen-presenting cells in the presence of dying/dead cells *in vitro* and to assess effectiveness of prophylactic vaccination with dying cells in the *in vivo* tumor models using syngeneic immunocompetent animals [13-15].

HMGB1 in neurodegenerative diseases. Hyperproduction and release of HMGB1 into the extracellular space is one of the important pathogenetic mechanisms in neurodegenerative diseases. Many factors associated with neurodegeneration could provoke the release of HMGB1, such as oxidative stress, hypoxia, inflammation, as well as traumatic injuries and viral infections [106, 121]. Disruption of the regulation of expression and activity of peroxiredoxins and, in general, of the oxidative-reductive balance contributes to the development of chronic HMGB1-induced low-level inflammation and is observed in almost all neurodegenerative diseases [122].

The HMGB1-mediated activation of glial cells, primarily microglia and astrocytes, in a neurotoxic manner leads to the excessive production of pro-inflammatory cytokines, chemokines, reactive oxygen species (including superoxide anions, hydrogen peroxide, hydroxyl radicals), and nitrogen species (NO), as well as proteolytic enzymes. This disrupts synaptic homeostasis, neuronal metabolism, leads to increase in the BBB permeability, and ultimately contributes significantly to the CNS pathology, promoting neuronal damage and accelerating neurodegenerative processes [123]. Moreover, neurodegenerative changes in AD and PD, including formation of protein aggregates of Aβ and α-synuclein, could, in turn, enhance neuroinflammation and additionally activate glial cells. Thus, a loop of positive feedback is formed, creating a self-reproducing and self-enhancing inflammatory environment [124]. Formation of such inflammatory environment is a universal feature of all neurodegenerative diseases.

In addition to the direct interaction with receptors, HMGB1 could enhance inflammatory processes by forming heterocomplexes with other DAMPs and pro-inflammatory molecules [125], including cytokines and chemokines [126-128], and mitochondrial DNA [129]. Formation of such complexes multiplies the inflammatory signal through the TLR receptors (including TLR3, TLR7, and TLR9), NF-κB, and complement pathways, leading to the enhanced activation of microglia with the neurotoxic M1 phenotype and disruption of the BBB integrity.

The BBB regulates transport of various molecules and ions from the bloodstream to nerve cells, preventing entry of pathogens and neurotoxins [121].

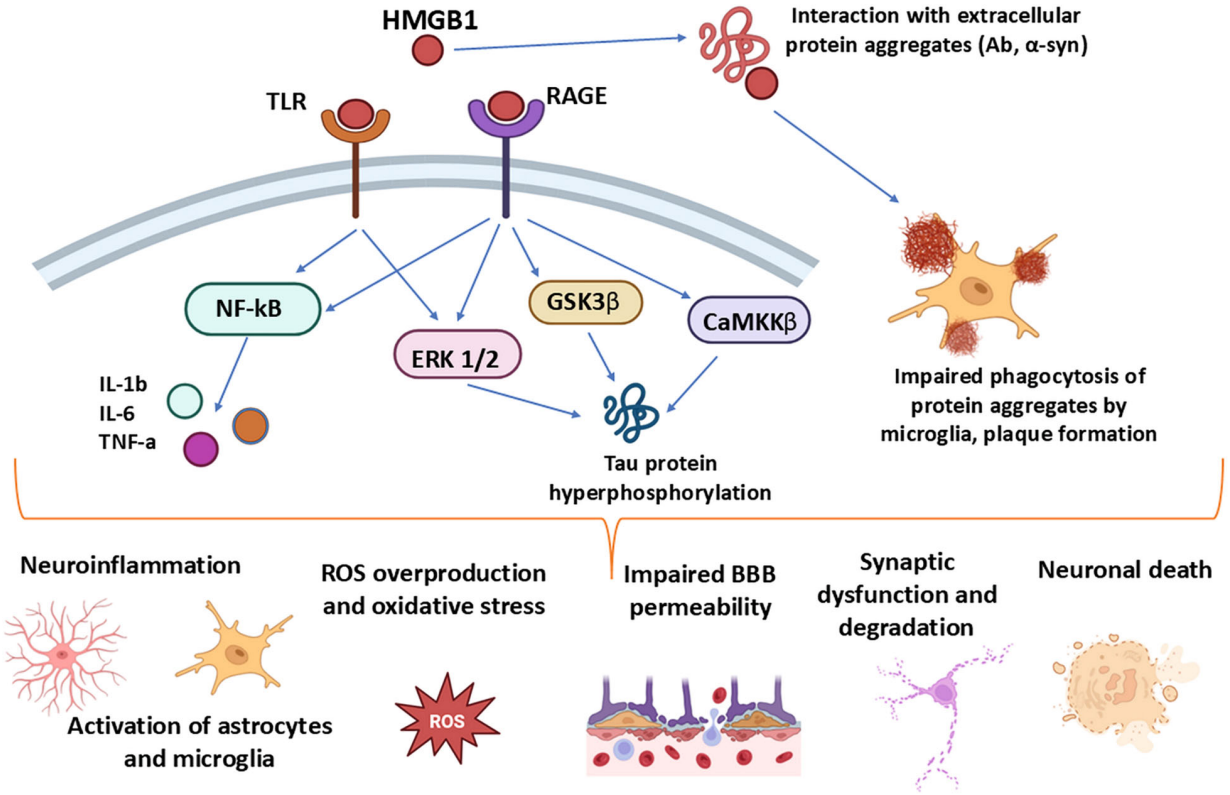

Fig. 3. Signaling pathways of HMGB1 in neurodegenerative processes. HMGB1 could interact with extracellular peptide aggregates (β-amyloid in AD or α-synuclein in PD), which leads to disruption of clearance of these proteins by microglia and their accumulation in the extracellular space. This causes synaptic dysfunction, development of neuroinflammation, and oxidative stress. Binding of HMGB1 to RAGE and TLR receptors (including TLR2, TLR4, TLR5, TLR9) induces NF-κB-mediated neuroinflammation, leading to increase in the production of pro-inflammatory cytokines, including TNF-α, IL-6, and IL-1β. In response to production of pro-inflammatory cytokines, activation of microglia and astrocytes with neurotoxic phenotype occurs, leading to formation of a vicious cycle of neuroinflammation and oxidative stress. Activation of the RAGE/TLR4-ERK1/2, RAGE/CaMKK-β-AMPK, and RAGE/GSK-3β pathways enhances production and accumulation of Aβ and abnormal hyperphosphorylation of tau protein. In addition, activation of the HMGB1/RAGE and HMGB1/TLR pathways disrupt integrity of the blood-brain barrier (BBB), which, in turn, enhances neuroinflammation. The resulting multiple positive feedback loops enhance neurodegenerative processes and lead to the death of both neurons and glial cells.

It should be noted that permeability of the BBB significantly increases during the development of neurodegenerative processes, which leads to disruptions in the homeostasis of the brain intercellular environment [130]. HMGB1 could increase permeability of the BBB by activating the TLR4 pathway and underlying signaling pathways NF-κB, Erk1/2, and Akt in the endothelial cells in a paracrine manner, as shown both in *in vivo* and *in vitro* models [131]. An important role in the disruption of the BBB permeability is played by degradation of the tight junction proteins, such as zonula occludens-1 (ZO-1) [132, 133]. The use of antibodies against HMGB1 reduces severity of brain edema and decreases permeability of the BBB in various pathologies, in PD in particular.

Interaction of HMGB1 with the protein aggregates formed in AD and PD, which disrupt normal functioning of the neurons and lead to their death, is of particular interest in the context of the development of neurodegeneration and inflammatory processes. It has been shown that HMGB1, by binding to the Aβ42 peptide – one of the key factors provoking AD – inhibits its phagocytosis by macrophages, as well as by microglial cells, and reduces clearance of the Aβ42 peptides introduced into the rat brain [134]. In the brain and serum of the AD patients, increase in the HMGB1 levels has been observed, which is probably one of the important causes of impaired clearance of amyloid peptides [133-135]. In addition, it has been shown that in the brain tissues of AD patients, HMGB1 accumulates in the Aβ plaques and in the surrounding microglial cells. In turn, both Aβ42 oligomers and pathological tau oligomers could activate the secretion of HMGB1 by microglia, sustaining the vicious cycle of neuroinflammation development [136].

The second mechanism by which HMGB1 stimulates production of the Aβ peptides is activation

of the RAGE receptor, which is expressed in microglial cells and neurons, and in AD, its expression is increased [137]. Hyperexpression of RAGE by the microglial cells enhances production and accumulation of Aβ42 in the hippocampus and cortex of the transgenic mice, increased severity of neuroinflammation, and contributed to the loss of cholinergic neurons [138]. The influence of RAGE on the Aβ42 production is associated with enhancement of amyloidogenic processing of the Aβ precursor by increasing activity of the β- and γ-secretases and GSK3β kinase [139]. A scheme summarizing signaling pathways activated by HMGB1 during the development of neurodegenerative processes is presented in Fig. 3.

In the postmortem studies of midbrain tissues, as well as of cerebrospinal fluid and serum of the PD patients, the elevated levels of HMGB1 were detected. It was established that the HMGB1 protein was located mainly in the nucleus of the cells in the healthy individuals and in the cytoplasm of the PD patients, which may indicate involvement of HMGB1 translocation in pathogenesis of the disease [140]. HMGB1 could bind to the aggregated α-synuclein and be present in Lewy bodies containing α-synuclein filaments isolated from the brain tissue [141]. It has been shown that the extracellular HMGB1-mediated activation of RAGE and TLR4 signaling pathways in PD accelerates the death of dopaminergic neurons and enhances inflammation in the substantia nigra, with excessive production and release of HMGB1 occurring already at the early stages of the disease [142]. It is worth noting that the endogenous intracellular cytoplasmic HMGB1, on the contrary, promotes degradation of α-synuclein through autophagy in the PC12 neuroblastoma cell line [143].

In the ALS patients, increased serum levels of the anti-HMGB1 autoantibodies were observed compared to the healthy individuals of the corresponding age [144]. At the same time, in the ALS patients and in the mouse models, nucleocytoplasmic translocation of HMGB1 was observed in the reactive astrocytes and microglia [145, 146]. Thus, HMGB1 could serve as a biomarker for diagnosis and clinical assessment of ALS. In ALS, as in other forms of neurodegeneration, HMGB1 could initiate a positive feedback loop, which, as shown both in humans and in animal models, exacerbates neuroinflammatory and neurodegenerative processes [145, 147].

In autoimmune neurodegenerative diseases, such as multiple sclerosis, HMGB1 activates the TLR4/NF-κB signaling pathway and induces pyroptosis in the microglial and other cells [148, 149]. The HMGB1-induced inflammation, in turn, contributes to demyelination. It has been suggested that increase in the HMGB1 levels in the cerebrospinal fluid, but not in serum, could be considered as a prognostic biomarker in multiple sclerosis [149].

Thus, HMGB1 plays an important role in the development of neurodegeneration, primarily through induction of neuroinflammatory processes, and, therefore, could be considered as a target for the therapy of neurodegenerative diseases. The main targeted approaches associated with HMGB1 include the use of neutralizing antibodies, low-molecular-weight antagonists (e.g., glycyrrhizin and ethyl pyruvate), competitive peptide antagonist BoxA, which binds to HMGB1 receptors and blocks signaling, as well as anti-inflammatory compounds of plant origin and cholinergic agonists [121]. Targeting HMGB1 has shown high efficacy in the experimental and preclinical models of AD, PD, ALS, and several other pathologies [121, 150], but clinical validation is practically absent. This direction requires further in-depth study.

CONCLUSION

Contrary to the classical paradigm of brain immune privilege, which has its own immune cells – microglia (Fig. 1) – and lacks peripheral immune cells, evidence has been accumulated of close interaction between the CNS and the immune system. The key links in this dialogue are DAMP molecules, which are widely represented in the brain, and their receptors are actively expressed not only by microglia, but also by astrocytes, neurons, endothelial cells, and infiltrating peripheral immune cells in the cases of vessel damage or BBB destabilization.

The key DAMPs reviewed in this article – CRT and HMGB1 – play multiple roles in the CNS. Under physiological conditions, they ensure normal functioning of the brain, including acting as necessary effectors of neurogenesis and homeostatic plasticity. When stress or a pathological stimulus (a “danger signal”) occurs, these molecules act as mediators between the dying cells and immune system, determining the pathways of immune response development: activation of sterile inflammation, induction of ICD, or, conversely, development of immunological tolerance.

Dysregulation, post-translational modifications, or excessive release of DAMPs into the tumor microenvironment or during neurodegenerative processes triggers a cascade of pro-inflammatory signals, exacerbating chronic neuroinflammation, disrupting integrity of the BBB, and forming resistance to therapy. Deep understanding of the mechanisms of conformational transformations of DAMPs, their concentration and kinetics of release, as well as features of their interaction with other DAMPs and formation of a complex pattern of danger molecules, becomes the key to controlling immune reactions in the CNS.

In this regard, detection of ecto-CRT and HMGB1 in the biological fluids or brain tissues could become an important prognostic tool, and regulation of the DAMP signaling pathways opens promising approaches for overcoming tumor resistance or inhibiting progressive neurodegenerative changes in the brain. The key challenge for future research is development of the strategies that allow selective modulation of the signaling pathways of CRT and HMGB1, while preserving their physiological functions. This would enable effective translation of the knowledge about the DAMPs into the safe therapy methods aimed at restoring homeostasis and immune surveillance in the CNS.

Abbreviations

Aβ	β-amyloid
AD	Alzheimer's disease
ALS	amyotrophic lateral sclerosis
ATP	adenosine triphosphate
BBB	blood-brain barrier
CNS	central nervous system
CRT	calreticulin
DAMP	damage-associated molecular pattern
ER	endoplasmic reticulum
HMGB1	high-mobility group box 1
ICD	immunogenic cell death
IL	interleukin
MHC I	major histocompatibility complex class I
PAMP	pathogen-associated molecular pattern
PRR	pattern-recognition receptor
RAGE (AGER)	receptor for advanced glycation end products
TLR	Toll-like receptor

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Contributions

T. A. Mishchenko, D. V. Krysko, and M. V. Vedunova: concept and supervision of the work, writing and editing of the article text. E. V. Mitroshina: literature search and analysis, writing and editing of the article text. S. S. Kulikov, A. A. Grechukhina, and O. J. Olajide: literature search and analysis, writing of the text, preparation of graphical material.

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Ethics approval and consent to participate

This work does not contain any studies involving human and animal subjects.

Conflict of interest

The authors of this work declare that they have no conflicts of interest.

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