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# PAD2-mediated citrullination of MBP and early myelin destabilization in multiple sclerosis

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ABSTRACT

Proteins affect most physiological functions in cells and play a key role in maintaining cellular homeostasis. Protein function is tuned at multiple levels, including synthesis and degradation, intracellular localization, and posttranslational modifications (PTMs). PTMs are a key chemical modification for regulating protein biological activity, including protein-folding relevant shifts in electrical charge, protein-protein interactions, and hydrogen bond formation. Such chemical changes can also result in protein unfolding or denaturation. PTMs can target proteins across multiple cellular compartments, including the cell membrane, cytoplasm, nucleus, endoplasmic reticulum, and mitochondria, playing crucial roles in regulating cellular processes, and their dysregulation is linked to the development of numerous diseases. It is well-established that various autoimmune conditions, including multiple sclerosis (MS), are influenced by the PTMs of endogenous proteins. This review adopts an integrated perspective on PTMs, with a particular focus on the citrullination of myelin basic protein (MBP). It moves beyond the traditional view of MS solely as an autoimmune disease, highlighting the broader implications of this initial chemical event, including the role of PAD2, in early disease onset and progression.

## 1. Introduction

Myelin formation is a highly regulated process that depends on the differentiation of oligodendrocyte progenitor cells (OPCs) into mature oligodendrocytes (OLs) and the subsequent wrapping of axons to generate a compact myelin sheath [1]. In multiple sclerosis (MS), a chronic neuroinflammatory and neurodegenerative disease of the central nervous system (CNS), disruption of myelin integrity and progressive axonal damage represent core pathological features affecting both white (WM) and grey matter (GM) [2,3]. Although MS has long been conceptualized as a primarily autoimmune disorder driven by peripheral immune activation ("outside-in" hypothesis), accumulating evidence indicates that myelin and OLs pathology may precede or occur independently of overt immune infiltration ("inside-out" hypothesis) [4–8]. Ultrastructural and molecular studies have revealed early abnormalities in normal-appearing white matter (NAWM), including subtle alterations in axon–myelin stability, lipid organization, $Ca^{2+}$ homeostasis, and OLs function, in the absence of demyelination or inflammation [5,9–12]. These observations suggest that early biochemical changes within myelin may be associated with lesion development and immune responses in MS. Myelin basic protein (MBP) is a central structural component of CNS myelin and is essential for its compaction and stability [13–16] (Figs. 1 and 2). MBP is an intrinsically

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**List of abbreviations**

**BBB**– Blood-brain barrier
**citMBP**– Citrullinated MBP
**CNS**– Central nervous system
**CSF**– Cerebrospinal Fluid
**cPLA2**– Cytosolic phospholipase A2
**DMTs**– Disease-modifying therapies
**EAE**– Experimental autoimmune encephalitis
**EBV**– Epstein–Barr virus
**EVs**– Extracellular vesicles
**GM**– Grey Matter
**LPC**– Lysophosphatidylcholine
**MBP**– Myelin basic protein
**MOG**– Myelin oligodendrocyte glycoprotein
**MS**– Multiple sclerosis
**NAWM**– Normal-appearing white matter
**NF-κB**– Nuclear factor kappa B
**NMDA**– N-methyl-D-aspartate
**OPCs**– Oligodendrocyte precursor cells
**OLs**– Oligodendrocytes
**PAD**– Peptidyl Arginine Deiminase
**PD**– Phosphatidylcholine
**PPMS**– Primary-progressive MS
**PTM**– Post-translational modification
**RRMS –** Relapsing-remitting MS
**ROS**– Reactive oxygen species
**SPMS**– Secondary progressive MS
**TTR**– Transthyretin
**WM**– White matter

disordered, highly basic protein that interacts electrostatically with negatively charged myelin lipids [14,15]. Its function is finely tuned by post-translational modifications (PTMs), which generate distinct charge isomers (C1–C8) with different biochemical and structural properties [17,18]. PTMs include phosphorylation, methylation, deamidation, ADP-ribosylation, N-terminal acylation, and citrullination [18] (Figs. 2 and 4; Table 1). Citrullination mediated by peptidyl arginine deiminases (PADs) is impactful, as it neutralizes positive charges on arginine residues, weakens lipid interactions, and destabilizes myelin structure [21, 37–40]. A growing body of evidence from human tissue, experimental models, and immunological studies indicates that citrullination of MBP is increased in MS and may contribute to myelin destabilization, impaired remyelination, and enhanced immune recognition of myelin antigens [5,21,37] (Tables 1 and 2). These findings raise the prospect that dysregulated citrullination may represent an early biochemical event linking myelin instability to immune activation in MS. Although MBP is the most extensively studied target of citrullination in MS, other myelin- and glia-associated proteins are also subject to this modification. Notable examples include glial fibrillary acidic protein (GFAP), myelin oligodendrocyte glycoprotein ($MOG_{35–55}$), myelin proteolipid protein (PLP), and vimentin. Proteomic analyses have also revealed that more than 80 proteins are citrullinated in brain tissue from MS patients [43], highlighting the pervasive impact of PAD-mediated citrullination across the CNS proteome. The aim of this review is first to introduce PTMs relevant to myelin biology. We then describe the enzymatic machinery responsible for protein citrullination, with a particular focus on PAD2. Next, we discuss the structural and functional consequences of MBP hypercitrullination, highlighting its potential role in the MS lesions, and emphasizing that, while PAD2-mediated citrullination may be an important contributor to MS pathology, it likely acts in concert with

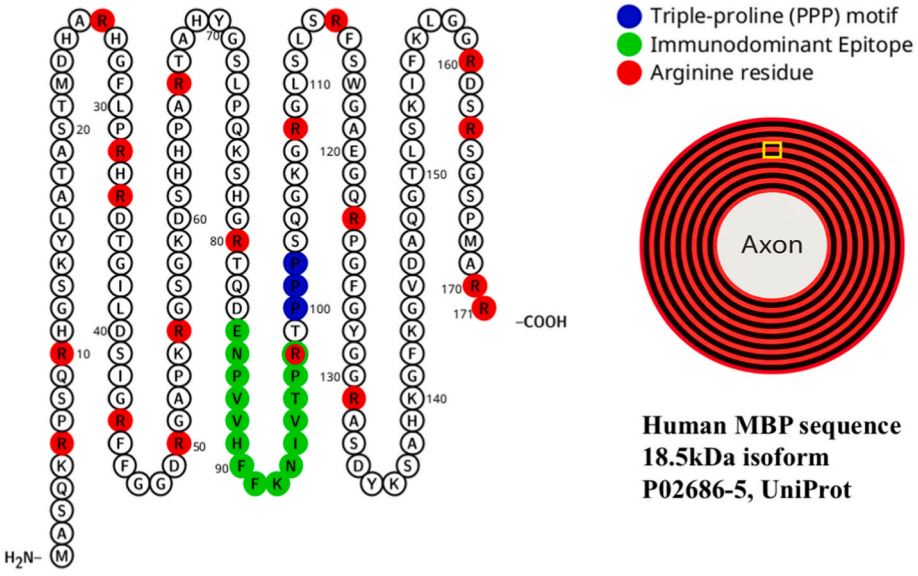


**Fig. 1. Structural and biochemical peculiarities of Myelin Basic Protein**
Schematic representation of the amino acid sequence of human MBP (18.5 kDa isoform; UniProt P02686-5) annotated with functionally relevant motifs. Arginine residues are highlighted in red, indicating potential sites for PAD-mediated citrullination. The triple-proline (PPP) motif, highlighted in blue, marks a region implicated in conformational rigidity and structural organization of this otherwise intrinsically disordered protein. The immunodominant peptide (ENPVVHFF-KNIVTPR) is highlighted in green.
The yellow square in the schematic myelinated axon cross-section denotes the hypothetical ultrastructural location of MBP shown enlarged on the left.
The amino acid sequence of human MBP (18.5 kDa isoform) was retrieved from UniProt (P02686-5), and used as reference. Residue numbering follows the UniProt sequence and may differ by one position from the conventional MBP85-99 peptide nomenclature used in the literature.
**Abbreviations:** MBP, myelin basic protein; PAD, peptidylarginine deiminase. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

other factors within a complex pathogenic process rather than as a single driver.

## 2. Post-translational modifications: from physiological regulation to pathological alterations in MS pathogenesis

Protein expression, binding, folding, activity, and stability are just a few of the biochemical characteristics impacted by PTMs [38,46,47]. Although these chemical substitutions represent an underappreciated source of critical data in biomarker research, they are not typically examined or explicitly sought in procedures. PTMs are increasingly recognized to provide relevant insights into disease mechanisms rather than serving solely as correlational biomarkers [38]. As proteomics has evolved, it has been discovered that PTMs, which significantly increase the complexity and diversity of proteomes, are hallmarks of all biological functions [38,48]. Covalent changes, including glycosylation, phosphorylation, acylation, citrullination, methylation, and ubiquitination, enhance the functional variety of proteins by conferring distinct structural properties, interaction capacities, and regulatory functions [38,48,49]. PTMs can arise in two primary ways: i) as regulated processes following transcription and translation of DNA into proteins, and ii) as responses to environmental stressors such as reactive oxygen and nitrogen species. In the first case, proteins are exposed to modifying enzymes (e.g., ligases, transferases, protein kinases) that alter their backbones or side chains, affecting structure and function, while in the second case, PTMs may intervene to preserve or restore proper protein function [38,46,50]. When aberrant PTMs appear, it is important to consider the existence of malfunctioning enzymes and/or alterations in the microenvironment within organelles or cells [38]. These events could lead to potential alterations in signaling pathways, pathogenic changes, and identification of novel disease biomarkers. With a key role in functional proteomics, PTMs have recently been associated with the onset and progression of demyelinating diseases, including MS [49]. Several studies investigating the role of PTMs in MS, which emphasize myelin proteins, particularly examining the structure of MBP from species such as bovine and dogfish [40,51], as well as humans [49], have shown the ability of several proteins to undergo unconventional PTMs, including β1-tubulin and β-actin from platelets and megakaryocytes [52] paving the way to identify novel diagnostic and prognostic biomarkers for MS. For instance, Kim et al. reported that PTMs of MBP including increased methylation and decreased phosphorylation in MS patients contribute to disease progression (Fig. 4) [49]. Considering these outcomes, the authors proposed to classify MS as a "*post-translational disease*," in which PTMs may contribute to the progression and heterogeneity of the illness. Another study by Salazar et al. analyzed the cerebrospinal fluid (CSF) proteome of MS patients with relapsing-remitting clinical forms (RRMS) and those of patients with other disorders of the CNS (both inflammatory and non-inflammatory), highlighting 13 differentially expressed protein spots across three patient groups, including transthyretin (TTR), apolipoprotein E, gelsolin, and alpha-1-antichymotrypsin. In particular, TTR-oligomerization was associated with a PTM-structural alteration specifically detected in MS CSF, being absent in other patient groups [53].

However, the concept of MS as a "post-translational myelin disorder" remains tentative. PTMs are ubiquitous; any diseases might be linked to aberrant PTM regulation, making the association between these changes and MS pathogenesis difficult to fully disentangle. Thus, while PTMs provide valuable mechanistic insights and biomarker potential, it is critical to avoid overextending their role without a clear mechanistic framework. While certain PTMs of MBP and other proteins may influence disease progression, they are unlikely to represent the sole pathogenic drivers of MS. Furthermore, reliable detection and quantification of PTMs remain significant challenges in clinical settings, limiting their immediate translational potential. It highlights the need for further studies to determine whether targeting MS-associated PTMs could become a therapeutic strategy.

### 2.1. Unconventional MBP post-translational modifications as mediators of MS pathogenesis

MBP is widely regarded as a candidate autoantigen, and autoantibodies against this protein have been considered potential contributors

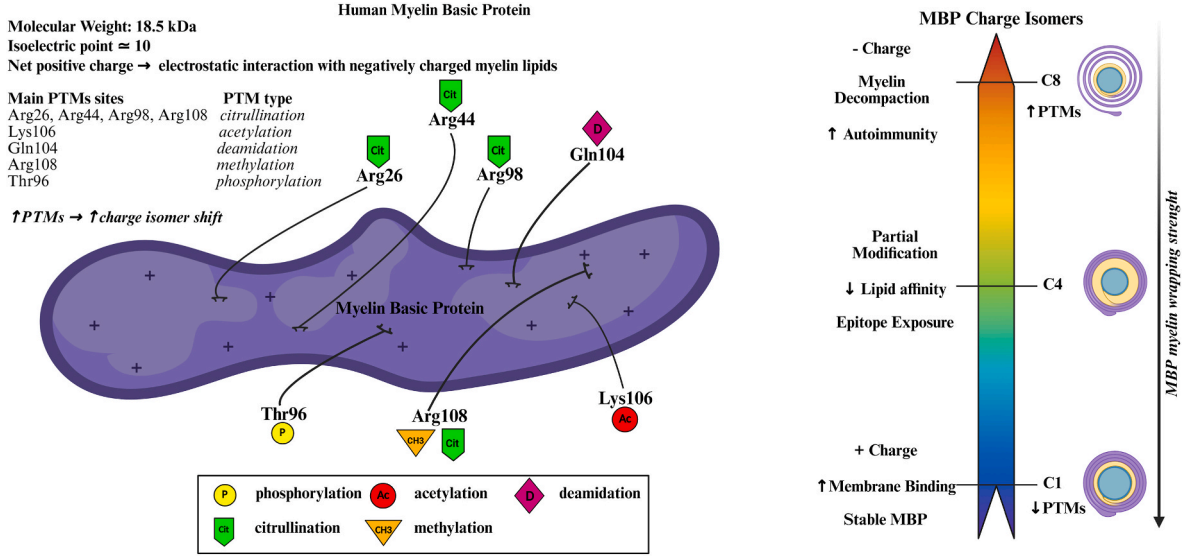


**Fig. 2. Post-translational modifications of Myelin Basic Protein and the generation of charge isomers (C1–C8).**
**Left panel:** Schematic representation of human MBP showing key PTMs, including citrullination, methylation, acetylation, phosphorylation, and deamidation, occurring at specific amino acid residues. These modifications modulate the physicochemical properties of MBP, especially its net positive charge, which is essential for electrostatic interaction with negatively charged myelin lipids.
**Right panel:** Conceptual gradient illustrating the spectrum of MBP charge isomers (C1-C8), where cumulative PTMs progressively reduce the basic nature of MBP. This transition, from highly basic (C1) to more acidic forms (C8), progressively reduces MBP membrane affinity and wrapping strength, and has been associated with increased epitope exposure and susceptibility to proteolysis.
**Abbreviations:** MBP, myelin basic protein; PTMs, post-translational modifications.

**Table 1**
General overview of the most significant post-translational modifications of MBP.

| PTM | Biochemical effect on MBP | Impact on MBP function and stability | Relevance to CNS Disorders | Study (Year) |
|---|---|---|---|---|
| **Citrullination** | Arg converted into Cit via PADs | ↓ Electrostatic binding to myelin lipids; loss of compaction | Enhances immunogenicity, microglial activation, and T cell responsiveness in MS | Martin et al. (1994) [19]<br>Tranquill et al. (2000) [20]<br>Moscarello et al. (2007) [21]<br>Oguz et al. (2009) [22]<br>Luchicchi et al. (2021,2024) [5, 23]<br>Muñoz González et al. (2025) [9] |
| **Phosphorylation** | Addition of a negative phosphate group to Ser/Thr residues | ↓ MBP's ability to tether to microtubules and lipid vesicles disrupts cytoskeletal interactions | Observed in MS; may promote MBP disassembly | Boggs et al. (2006) [24]<br>Boggs et al. (2011) [25]<br>Boggs et al. (2012) [26] |
| **Deamidation** | Hydrolytic removal of the amide group, converting Asn/Gln into acidic residues (Asp/Glu) | Alters charge distribution; may destabilize structure, affect the stability of the myelin sheath, and expose hidden epitopes | Observed in MBP from the human brain; role in demyelinating disorders remains unclear | Sarg et al. (2017) [27]<br>Adav (2025) [28] |
| **Acetylation** | Acetyl group from acetyl-CoA covalently binds ε-amino group of Lys residues | May weaken lipid membrane interactions and myelin compaction, potentially increasing MBP solubility | ↑ Lys acetylation observed at the peak of neurological disability in EAE, suggesting a potential involvement in demyelinating processes | Zhang et al. (2012) [18]<br>Lillico et al. (2018) [29] |
| **Methylation** | Introduction of mono- and dimethyl groups on Arg residues | Modulates MBP folding and influences protein-lipid interactions | While physiologically protective, aberrant methylation patterns may enhance immunogenicity in demyelinating disorders | Kim et al. (1997) [30]<br>Pritzker et al. (2000) [31]<br>Zhang et al. (2012) [18] |
| **ADP-ribosylation** | Covalent addition of ADP-ribose to Arg residues | Modulates the charge profile of specific MBP isoforms; potential implications for membrane interaction remain to be clarified | Induced in vitro in human and murine myelin. No physiological or pathological role has been demonstrated up to date | Boulias and Moscariello (1990) [32]<br>Boulias and Moscariello (1994) [33]<br>Boulias et al. (1995) [34] |
| **N-terminal acylation** | Covalent addition of short-chain acyl group to the protein's N-terminal | May alter MBP charge isomer profile and modulate immunoreactivity | Observed in human and bovine MBP. Functional relevance in demyelinating disorders remains uncertain | Moscarello et al. (1992) [35]<br>Zhou et al. (1993) [36]<br>Zhang et al. (2012) [18] |

Table 1. General overview of the most significant post-translational modifications of MBP, along with their known or suspected biochemical effects and possible connection to demyelination of the CNS. Emphasis is placed on modifications that affect MBP's charge, hydrophobicity, or structural conformation, which may impair its capacity to maintain myelin compaction or promote the exposure of immunogenic epitopes. Other modifications such as deamidation, phosphorylation, acetylation, and N-terminal acylation have also been described and may contribute to MBP immunogenicity, even though citrullination is the PTM that has been studied the most in relation to MS and other demyelinating disorders. Evidence was derived from both in vitro and in vivo studies in human and animal models. This table offers a comprehensive overview of MBP PTMs, while MBP citrullination is addressed as the central topic of the review in Table 2.

to MS pathophysiology [17,18,21,49]. Although genomic studies have not identified a direct mechanism underlying this phenomenon, research investigating MBP abnormalities in MS has increasingly focused on the potential contribution of PTMs [17,49], particularly given the propensity of MBP to undergo extensive PTMs [18,49] (Figs. 2 and 4; Tables 1 and 2). These modifications modulate MBP interactions with myelin membranes and other myelin-associated proteins. This fits into a broader framework in which citrullination has emerged as a widely occurring PTM across diverse physiological and pathological conditions, as demonstrated by a recent quantitative proteomic mapping study [54]. Because MBP has been associated with demyelinating disorders [15,17,49,55], it has been hypothesized that unconventional PTMs may increase its antigenicity [45,49]. Citrullination of MBP, which reduces the protein's positive charge required for compact myelin organization, likely contributes to MS pathogenesis through two complementary mechanisms: (i) structural destabilization of the myelin sheath and (ii) increased autoantigenicity. In the study by Zhang et al., unconventional PTM patterns were also reported that may contribute to demyelination, altered antigenicity, and disrupted interactions with phospholipid membranes [18]. While these correlations support the hypothesis that PTMs influence MS pathology, a direct causal relationship remains unproven. It is also plausible that abnormal isoform compositions or misfolding of MBP weaken membrane interactions and compromise the structural organization of the myelin lipid bilayer, leading to dysmyelination rather than demyelination [56], as observed in the leukodystrophy vanishing WM disease [57]. Such structural defects could also account for the presence of MBP in CSF and for detected anti-MBP immune responses. Overall, current findings support the concept that PTMs modulate MBP antigenicity and may influence MS onset. However, they also raise the possibility that MBP functions not solely as a passive autoimmune target (e.g., for autoreactive T cells and autoantibodies) but also as an active mediator in disease progression. This dual role underscores the need for more comprehensive research strategies to define how PTMs initiate immune responses and myelin

**Table 2**
MBP citrullination as a pathogenic modification in MS: key preclinical and clinical evidence.

| Study (Year) | Model | Main Findings | Relevance to MS Pathogenesis |
|---|---|---|---|
| **Martin et al. (1994)** [19] | Human T cell lines | T cells recognize citMBP peptides over native | Evidence of altered antigenicity due to citrullination |
| **Moscarello et al. (2007)** [21] | Human MS post-mortem brain | Elevated citMBP in MS brains vs controls; PAD-2 overexpression | Demonstrates increased citrullination of MBP and altered charge isomer distribution in human MS post-mortem brains. |
| **Wood et al. (2008)** [41] | PAD2 knockout mice; Human post-mortem brain | PAD2 accounts for the majority of MBP citrullination, with partial compensation by PAD4 in PAD2 knockout mice | Identifies PAD2 as a major enzyme responsible for citrullination of MBP, in both experimental mouse models and human post-mortem brains |
| **Oguz et al. (2009)** [22] | Human MS brain via proton MR spectroscopy | Citrulline peaks more frequently detected in the brains of patients with early-onset MS | Suggests that increased citrullination of myelin proteins is associated with demyelination in MS |
| **Moscarello et al. (2013)** [42] | Murine models of neurodegenerative and autoimmune demyelination | PAD inhibitor 2-chloroacetamidine reduces MBP hypercitrullination | Shows that pharmacological inhibition of PAD reduces MBP hypercitrullination and is associated with reduced demyelination in experimental models of MS |
| **Faigle et al. (2019)** [43] | Human post-mortem brain; $CD4^+$ T cells | Multiple citrullination sites mapped; higher levels in MS | Proteomics confirms citMBP enrichment in MS brains |
| **Standiford et al. (2021)** [44] | Cuprizone mouse model | CitMBP impairs remyelination via microglial TNFα activation | Reports increased citrullination of MBP together with microglial activation and reduced remyelination in cuprizone-intoxicated mice |
| **Monreal et al. (2023)** [45] | PBMCs from MS patients and healthy donors | Citrullination of MBP enhances secretion of TNF-α by T cells from patients carrying the MS-associated HLA-DR15 haplotype. | Suggests citMBP contributes to breach of tolerance and maintenance of autoimmune response in MS via enhanced antigen presentation |
| **Luchicchi et al. (2024)** [23] | Human MS post-mortem brain | Identification of a new histologically defined early phase in MS lesion formation, namely mDAWM, showing increased MBP citrullination | Suggests that increased citMBP, myelin blistering, and microglial response in NAWM are associated with mDAWM, consistent with early pathological events in MS |

Table 2. An overview of key preclinical and clinical studies investigating the role of MBP citrullination in MS. According to these studies, MBP hypercitrullination is associated with altered antigenicity, myelin destabilization, impaired remyelination, and increased microglial activation and T cell responsiveness. Taken together, these findings support the hypothesis that citrullination of MBP is associated with myelin instability and immune dysregulation in MS.

breakdown. One compelling example relates to Epstein–Barr virus (EBV), which is epidemiologically linked to MS [58], although the causal mechanism remains under debate. EBV has been implicated in altering PTMs [59], suggesting that deeper insights into MBP-related PTMs may illuminate EBV–MS interactions and identify therapeutic opportunities. While multiple PTMs regulate myelin protein function, the following sections focus specifically on MBP citrullination due to its unique structural, immunological, and pathological relevance to MS. Collectively, these observations suggest that MBP could be more than a passive target of autoimmunity; its PTMs, particularly citrullination, may actively shape both myelin integrity and immune activation, highlighting a critical intersection of structural and immunological mechanisms in MS. Clarifying these mechanisms may reshape our understanding of MS pathogenesis and provide new investigative routes.

## 3. PAD-mediated citrullination: predominant role of PAD2

### 3.1. PAD2-mediated citrullination and autoimmune recognition in MS

Citrullination, also known as deimination, is a PTM that converts peptidyl-arginine residues into peptidyl-citrulline [39,60,61] (Fig. 3). This $Ca^{2+}$-dependent reaction is catalyzed by PADs [62] and results in the loss of a positive charge, thereby altering protein structure, intermolecular interactions, and immunogenicity. Among the five human PAD isozymes, PAD2 and PAD4 are the most prominently distributed in OLs, astrocytes, microglia, neurons, and neural stem cells [42,62]; however, PAD2 is frequently cited as the predominant citrullinating enzyme in the OL lineage, responsible for the conversion of arginine to citrulline in both nuclear and cytoplasmic proteins [63]. Dysregulated citrullination has been implicated in a wide range of autoimmune, inflammatory, and neurodegenerative diseases beyond MS [64–66], largely driven by PAD2 hyperactivation. These alterations increase MBP antigenicity through PAD2-mediated citrullination, promoting its recognition by autoreactive T cells [66,67]. Experimental and clinical studies indicate that PAD2 activity modulates $CD4^+$ T-cell responses, promoting the activation and polarization of pro-inflammatory Th17 phenotypes that display heightened reactivity to citrullinated MBP epitopes [45,68–70].In addition, $CD4^+$ T cells from MS patients display increased reactivity toward citrullinated MBP epitopes compared with native MBP, indicating enhanced antigenic recognition following deimination [20]. Citrullination may also modulate pro-inflammatory signaling pathways, including TNF-α production in immune cells in PAD2-dependent contexts [45]. In this setting, citrullination generates neoantigens that alter MBP structural properties and increase its immunogenic potential, thereby linking post-translational modification to adaptive immune activation [20,45]. Importantly, in a hybrid cuprizone–autoimmune encephalitis model, inflammatory demyelination was attenuated by PAD2 inhibition [37], despite cuprizone-induced myelin injury [71], supporting a functional link between PAD2 activity and immune-mediated pathology. As reported in Section 3.3, evidence across MS studies is heterogeneous, and PAD2 overexpression has not been uniformly detected across all MS lesions. Nevertheless, multiple lines of evidence suggest that dysregulated PAD2 activity may contribute to early myelin pathology and increase the immunogenic potential of myelin-derived epitopes [37,41,42].

### 3.2. $Ca^{2+}$-dependent PAD2 activity: A functional switch between myelination and demyelination

PAD2 exerts critical role in CNS biology [60,61,63]. In physiological conditions, PAD2 expression increases upon OL differentiation, peaking in mature cells to facilitate myelination and chromatin remodeling [41,

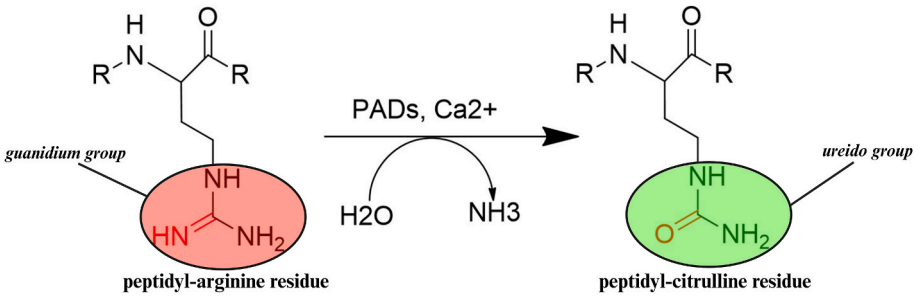


**Fig. 3. PAD-mediated citrullination of arginine residues.** Schematic representation of the $Ca^{2+}$-dependent PTM catalyzed by PADs, in which a peptidyl-arginine residue is converted into peptidyl-citrulline. The guanidinium group of arginine, positively charged due to resonance delocalization, is converted into a neutral ureido group through hydrolytic citrullination. This PTM results in the loss of a positive charge and alters the electrostatic profile of MBP, which may reduce its membrane-binding capacity and contribute to the altered structural and immunological properties.
**Abbreviations:** MBP, myelin basic protein; PTMs, post-translational modifications; PAD, peptidylarginine deiminase.

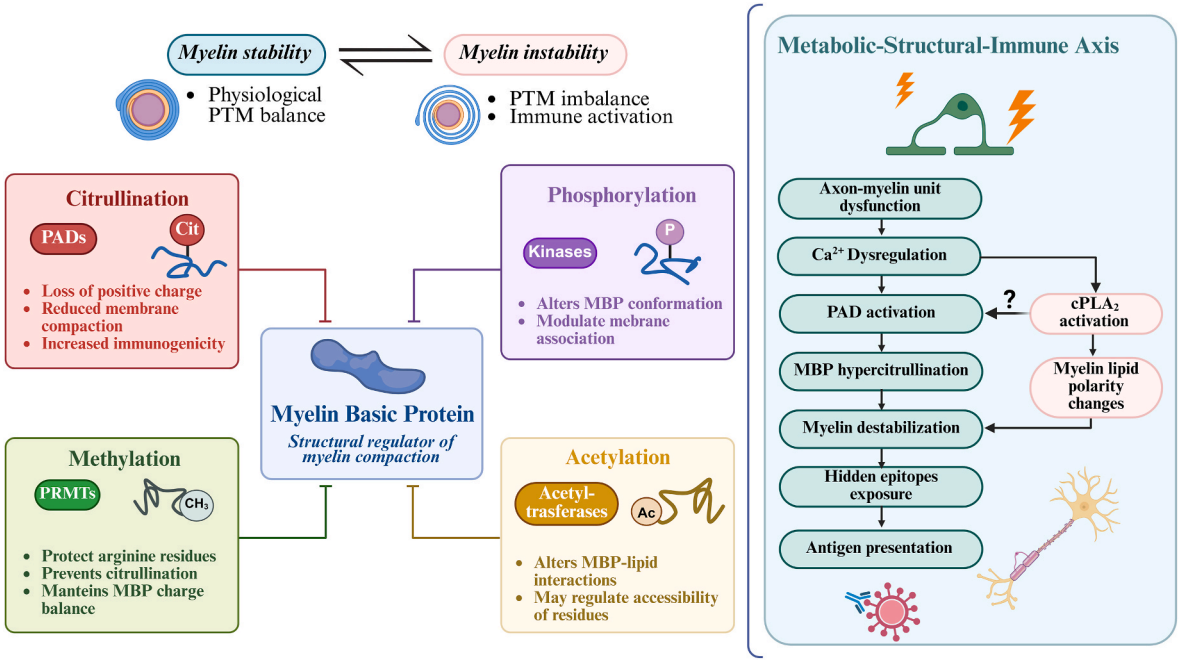


**Fig. 4. Crosstalk among post-translational modifications of Myelin Basic Protein and their potential impact on myelin stability and immunogenicity in multiple sclerosis.** Schematic representation of the PTM crosstalk regulating MBP stability and function. A balanced PTM pattern ensures myelin stability; however, PTM imbalance can lead to myelin destabilization and immune activation. The right panel illustrates a proposed pathogenic axis according to the inside-out hypothesis of MS: dysfunction of the axon-myelin unit and $Ca^{2+}$ dysregulation lead to PAD activation and hypercitrullination of MBP. This contributes to myelin destabilization, exposure of hidden epitopes, and antigen presentation. Furthermore, activation of $cPLA_2$ can alter myelin lipid composition, exacerbate structural and immunological alterations and reducing the activation threshold of PADs.
**Abbreviations:** MBP, myelin basic protein; PTM, post-translational modification; PAD, peptidylarginine deiminase; PRMT, protein arginine methyltransferase; $cPLA_2$, cytosolic phospholipase $A_2$.

63]. In this context, citrullination supports structural plasticity and myelin maintenance without compromising membrane integrity [41, 63]. However, PAD2 enzyme targets multiple substrates, including myelin-associated and chromatin-related proteins [63],underscoring its involvement in both structural myelin integrity and epigenomic regulation, in coordination with other PTMs [63]. Of note, PAD2 activity is strictly $Ca^{2+}$-dependent, requiring intracellular $Ca^{2+}$ concentrations approximately 100-fold higher than basal cytosolic levels ($10^{-8}$–$10^{-6}$ M) and $Ca^{2+}$ signaling has been shown to be fundamental for OPCs migration, differentiation, and myelination [72]. $Ca^{2+}$ binding induces conformational rearrangements that generate the active site within the C-terminal domain, while additional N-terminal $Ca^{2}$-binding sites stabilize the enzyme and ensure full activation [62,73]. In MS context, altered $Ca^{2+}$ homeostasis in axons and OLs mediated by N-methyl-D-aspartate (NMDA) receptors and voltage-gated $Ca^{2+}$ channels [5] drive excessive intracellular $Ca^{2+}$ influx, dysregulated PAD2 activation and promoting hypercitrullination of MBP [74]. The result consists in a reduction of MBP net positive charge, weakening its interaction with negatively charged myelin lipids and promoting myelin destabilization (Fig. 2). This process is proposed to exacerbate demyelination, axonal damage, and fragmented citMBP becomes more susceptible to proteolytic processing, increasing the generation of neo-epitopes with enhanced immunogenic antigen presentation to autoreactive T cells already present in the NAWM of MS patients. Together, these processes illustrate a context-dependent dual role of PAD2, in which physiological activity supports myelin homeostasis and repair, whereas its dysregulation drives structural vulnerability and immune-mediated demyelination. Transcriptomic and biochemical studies indicate increased PAD2 activity in lesion areas and NAWM [5, 23], although PAD2-mediated MBP citrullination in experimental

models may precede overt demyelination. Its relevance in human MS remains unresolved. Importantly, PAD2-mediated citrullination of MBP has been shown to precede extensive myelin breakdown and the onset of clinical symptoms, suggesting a potential early contribution in experimental systems, although its temporal role in human MS remains unresolved. In MS tissue, citrullinated MBP (citMBP) is enriched in WM and associated with epigenetic regulation, including hypomethylation of the PAD2 promoter, which increases PAD2 expression and activity [75]. This spatial and temporal variability may explain discrepancies across studies. Beyond myelin structure, PAD2 regulates chromatin accessibility and OL lineage progression [63]. Its inhibition impairs chromatin remodeling and reduces expression of OL differentiation genes, while PAD2-knockout mice exhibit motor deficits and a significant reduction in myelinated axons within the corpus callosum, a region highly relevant to MS pathology [41,63]. Overall, PAD2 functions as a context-dependent regulator of myelination, with therapeutic strategies potentially benefiting from modulation of activity rather than complete inhibition, including targeting $Ca^{2+}$ signaling or PTM balance. This framework describes PAD2 as a "diverse functional switch" and underscores that therapeutic strategies should aim to fine-tune, rather than completely inhibit, PAD2 activity by targeting $Ca^{2+}$ signaling, enzymatic activation, or the balance between citrullination and competing PTMs (e.g. arginine, methylation) to preserve its physiological role in myelination while preventing pathological hypercitrullination.

### 3.3. Uncoupling between PAD2 expression and activity: implications for MBP citrullination dynamics

Several studies report discrepancies between PAD2 expression levels and the degree of MBP citrullination observed in MS tissue. PAD2 mRNA levels are significantly lower in the NAWM compared to control WM and lesional areas despite increased levels of citMBP [75]. This discrepancy suggests that PAD2 enzymatic activity may not directly correlate with elevated citrullination during early MS, raising the possibility of an uncoupling between expression and activity. Such uncoupling may be driven by altered intracellular $Ca^{2+}$ dynamics that enhance enzyme activation [76], differential transcriptional regulation versus PTMs [77], and the release of active PAD enzymes from damaged OLs [63]. In addition, endogenous inhibitors may further modulate PAD2 activity independently of its expression levels [78,79]. Together, these observations underline that PAD2-mediated citrullination of MBP is not an isolated event, but rather part of a complex and tightly regulated PTMs network (Fig. 4), which also includes phosphorylation, methylation, and acetylation [18,80,81]. Arginine methylation, for example, may protect specific MBP residues from PAD-mediated conversion because methylated arginines cannot be deiminated [49,82]. Phosphorylation [24,26,83] or acetylation [29] may influence MBP conformation and its interaction with the myelin membrane, thereby altering accessibility of arginine residues to PAD enzymes. Acetylation may alter MBP lipid interactions and influence the accessibility of arginine residues to PAD enzymes [29]. These observations underscore and reinforce two critical concepts: first, MBP function and stability are governed by a dynamic PTM crosstalk network, in which PAD2-mediated citrullination represents one of several coordinated regulators shaping myelin architecture and immunogenicity. Second, PAD2 activity operates along a functional continuum, ranging from physiological roles in myelination to pathological hyperactivation in disease.

### 3.4. Spatial and temporal heterogeneity of PAD2 activity: contrasting evidence and Alternative interpretations

In physiological conditions, citrullination supports OPC maturation and myelin repair, whereas excessive or sustained PAD2 activity contributes to structural myelin damage and immune exposure in MS [60,61,63,64]. However, recent evidence suggests for a more cautious and integrative interpretation of PAD2 role, emphasizing its context-dependent roles across MS lesion stages, clinical forms, and cellular environments [66,84]. Analyses of MS tissue do not uniformly report increased PAD2 expression, highlighting substantial heterogeneity across lesion stage, disease subtype, and neural cell populations [41,84]. While citMBP is predominantly detected in areas of active demyelination and chronic active lesions [85], PAD2 upregulation is more variably associated with disease progression [41,86]. Interpreting PAD2 and citMBP therefore requires moving beyond a purely descriptive view of heterogeneity toward a mechanistic, context-dependent framework reflecting distinct pathogenic programs rather than stochastic variability. In NAWM, increased citMBP in the absence of overt immune infiltration suggests that citrullination may precede classical inflammation [5]. However, rather than acting as a universal initiating factor, PAD2 activity is more plausibly an early permissive mechanism that destabilizes myelin and increases its susceptibility to subsequent immune-mediated damage. In active lesions, elevated PAD2 expression and activity contribute to myelin destabilization and increased immunogenicity, whereas in chronic or slowly expanding lesions, sustained activity at the lesion rim likely reflects ongoing contributions from microglia and astrocytes [67]. Kim et al., showed that anti-PAD2 autoantibody levels, used to track PAD2 activity, vary by MS subtype, with significantly higher levels in RRMS and secondary-progressive SPMS compared to primary progressive MS (PPMS) [84]. This pattern aligns with transcriptomic evidence indicating that peptidyl arginine deiminase 2 (PADI2) expression, that encodes PAD2 enzyme, may be elevated in RRMS but reduced in SPMS [87], consistent with a transition from inflammation-driven pathology toward a more neurodegenerative process. A mechanistically plausible explanation relates to the irreversible nature of citrullination, that leads to the accumulation of long-lasting molecular alterations contributing possibly to disease progression independently of ongoing inflammation. A critical aspect in interpreting these findings is the uncoupling between PAD2 expression and enzymatic activity. PAD2 function is primarily regulated by intracellular conditions, particularly $Ca^{2+}$ dynamics, metabolic state, and subcellular localization rather than transcriptional levels alone. This explains apparent discrepancies such as high MBP citrullination in regions with relatively low PAD2 expression and underscores the importance of cellular stress and OL dysfunction as key drivers of enzymatic activation. Importantly, as mentioned in section 3.3, PAD2-mediated citrullination does not occur in isolation but is embedded within a complex PTM network (Fig. 4). The concept that PTMs are not merely additive, but collectively determine site-specific susceptibility, aligns with the framework of "citrullinome" mapping. The presence of these modifications can alter protein conformation, creating a combinatorial "code" that modulates the accessibility of specific arginine residues to PAD2. Accordingly, pathological outcomes are more likely to arise from a breakdown in this coordinated PTM crosstalk than from a simple increase in enzymatic activity. While traditionally associated with OLs and OPCs, PAD2 is also highly expressed in astrocytes and other glial populations [88,89], where its activity may exert both protective and detrimental effects depending on the microenvironment. This reinforces the concept that PAD2 dysregulation is not uniform but shaped by lesion stage, metabolic context, and cellular composition. Overall, PAD2/-citMBP dynamics in MS reflect localized, environmentally regulated enzymatic activity within a broader PAD2–citrullination axis, where citrullination is highlighted as a key component of this system, where metabolic stress, $Ca^{2+}$ imbalance, and intercellular interactions converge to shift myelin from a stable to a vulnerable state. These considerations underscore the importance that PAD2/citMBP dynamics reflect spatially and temporally regulated enzymatic activity within a broader PTM and metabolic network governing myelin stability.

## 4. Integrating structural and immune consequences of MBP citrullination in MS pathogenesis

### 4.1. Structural vs immune contributions of MBP citrullination: cause, consequence, or feedback loop?

Building on the overview of PTMs and PAD2, this section examines how aberrant MBP citrullination may contribute to myelin destabilization and immune recognition. Myelin compaction is an MBP-dependent process [15,90]. Studies have shown that MBP is essential for maintaining myelin compaction, as demonstrated by conditional knockout models exhibiting progressive decompaction and shiverer-like phenotypes [16,90,91]. These findings confirm the central structural role of MBP, although they do not imply that continuous de novo synthesis is required under steady-state conditions [16,90]. Human MBP (P02686-5, MBP-3, 18.5 kDa isoform) contains 19 arginine, most of which are susceptible to citrullination [43]. Proteomic analyses showed that 16 out of 19 arginine sites were citrullinated in both the control and MS brains [43]. Under physiological conditions, ~20% of MBP is citrullinated, increasing to ~45% in SPMS and up to 90% in fulminant MS [43]. As outlined in the previous sections, citrullination converts positively charged arginine residues into neutral citrulline, reducing MBP's net cationic charge [49,61] (Figs. 2 and 3). This reduction weakens electrostatic interactions with negatively charged phospholipids, which are essential for membrane adhesion and myelin compaction [15,17,92]. In pathological conditions, this process reduces the cationic charge of MBP, weakens its binding to negatively charged phospholipids, promotes myelin destabilization, and alters axon–myelin communication. [93]. Beyond its structural consequences, citrullination of MBP is implicated in modulating adaptive immune responses [20,45,68]. Studies in MS patients have shown that increased PAD2 activity is associated with elevated citMBP, resulting in the generation of neoepitopes, destabilization of myelin sheaths, and increased susceptibility to recognition by the HLA-DR15 haplotype [20,45,94,95]. Specifically, the citrullinated form of the immunodominant peptide $MBP_{85\text{-}99}$ binds to HLA-DR15 and, in competitive settings, outcompetes the native, non-citrullinated MBP peptide potentially contributing to loss of immune tolerance and sustained autoimmunity [45,95]. The increased binding of citMBP peptides to HLA-DR15 and subsequent demyelination may be mechanistically linked to elevated hyperdynamic PAD2-mediated processes throughout the clinical phases of MS, which is characterized by highly inflammatory, relapsing activity in early stages that evolves into chronic, smoldering neurodegeneration in later progressive stages. A stage-dependent mechanistic scenario can be proposed. In inflammatory phases, such as RRMS, elevated PAD2 activity promotes citrullination of arginine residues within MBP. This leads to the loss of positive charge and improved accommodation of these peptides within the HLA-DR15 binding groove by adopting a linear conformation of MBP [95]. This results in more stable neo-antigen citrullinated peptide-MHC (pMHC) complexes and enhanced presentation of neo-epitopes to autoreactive $CD4^+$ T cells. Over time, given the irreversible nature of citrullination in humans, the elevated hyperdynamic PAD2 activity may lead to the accumulation of persistently citMBP within MS lesions. Even as PAD2 expression declines in later stages such as SPMS, the interplay between persistent PAD2 activity and preexisting citMBP may amplify autoreactive immune recognition and affect tissue integrity, contributing to a shift from inflammation-driven pathology toward neurodegenerative mechanisms. In this context, it is important to stress that citrullination is a physiological process and does not inherently trigger immune activation [60,61]. Furthermore, defective clearance of apoptotic or necrotic cells may amplify this process by promoting the release of PAD enzymes and citrullinated peptides into the extracellular space, enhancing immune exposure and perpetuating inflammation [96]. Importantly, most mechanistic insights derive from experimental models, and caution is required when extrapolating these findings to human MS as will discussed in section 4.2.

### 4.2. Evidence from experimental models and human studies: converging or diverging?

Evidence for a central role of citrullination of MBP at the origin of inflammatory demyelination stems from a novel experimental hybrid between cuprizone autoimmune encephalitis (CEA) and EAE, in which selective inhibition of PAD-mediated citrullination of MBP before immune stimulation could prevent inflammatory demyelination [37]. Contrastingly, inhibition of PAD after the onset of immune activity only offered minimal protection, further supporting a role for MBP citrullination in facilitating inflammatory demyelination in MS [37]. In the cuprizone-intoxication model, Caprariello et al. demonstrated that extensive MBP citrullination occurs early and is associated with early myelin alterations preceding overt immune activation in this model [37]. In ND4 transgenic mice, where PAD2 is overexpressed under the neurofilament light promoter, PAD2 mRNA and protein levels rise as early as two months of age, followed by increased MBP citrullination at three months [97]. Importantly, these molecular alterations occur before both demyelination and the onset of clinical symptoms, supporting a model in which PAD2 overexpression may act as an early driver of demyelination rather than a secondary consequence of neurodegeneration. Beyond its structural role, MBP peptides have recently been identified as prominent components of the CNS regulatory peptidome [98]. Comprehensive mapping of CNS-enriched autoantigenic peptides across the murine brain, leptomeninges, and dura mater revealed a notable abundance of MBP-derived epitopes within the naturally presented antigenic repertoire [43,98,99]. Importantly, after EAE induction with $MOG_{35-55}$, administration of native $MBP_{160-175}$ peptide or MBP-loaded extracellular vesicles (EVs) conferred immunosuppression and reduced CNS autoimmunity [100,101]. In contrast, EVs loaded with citrullinated $MBP_{160-175}$ did not confer protection, indicating that citrullination abolishes the immunomodulatory function of MBP and disrupts self-tolerance. These results provide mechanistic plausibility for a scenario in which biochemical destabilization of myelin may precede or amplify immune recognition within a controlled model system. However, current evidence does not support a strictly linear model in which citMBP simply precedes immune activation. In MS citMBP is highly enriched in WM, potentially because of PAD2 promoter hypomethylation leading to increased PAD2 transcription [42,75,86,97]. In regions of subtle demyelination or the absence of overt immune reactivity, citMBP levels are even higher than in the NAWM [23], suggesting a potential role in early lesion-associated protein modification. However, whether elevated MBP citrullination is directly driven by increased expression represents a reparative response or contributes to disease progression remains uncertain. In MS brain, increased citrullination has been observed in NAWM and prelesional regions [5,21,23], but these observations are largely based on postmortem correlative data and cannot determine whether citrullination precedes, follows, or co-evolves with inflammatory responses in vivo. Thus, while MBP citrullination may represent an early event in experimental models, its temporal relationship in humans remains to be determined. From this perspective, MBP citrullination should be viewed as a plausible early contributor to myelin vulnerability rather than a definitively established primary trigger of disease. In EAE model, citrullinated peptides alone are insufficient to initiate disease but significantly exacerbate pathology in the presence of an established autoimmune response, supporting a role for citrullination in disease amplification rather than initiation [96]. *In vitro* experiments MS patients demonstrated enhanced T-cell proliferation and responsiveness to citMBP [43]. These results provide mechanistic plausibility for a scenario in which biochemical destabilization may precede immune recognition. However, current evidence does not support a strictly linear model. Rather, biochemical destabilization, altered antigen presentation, and immune responses likely interact dynamically in a self-reinforcing loop. In this view, citMBP functions both as a modifier of myelin stability and as an amplifier of immune recognition, with relative contributions varying by disease stage.

Together, these observations argue against a unidirectional causal model and instead support a context-dependent contribution of citrullination within a multifactorial disease framework.

### 4.3. Context matters: lesion stage, regional heterogeneity, and disease dynamics

Immunoelectron microscopy localized PAD2 to intact mouse optic nerve myelin, with prominent clustering in periaxonal regions [41]. MBP hypercitrullination may contribute to localized myelin destabilization, causing the patchy degeneration characteristic of MS. This observation raises the possibility that myelin alterations may originate in the periaxonal region, contrasting with the autoimmune hypothesis of external myelin attack. An epigenetic study reported that PAD2 promoter hypomethylation may increase localized citrullination of MBP, while the presence of enzymatic machinery within myelin supports the possibility of locally regulated protein modification, and myelin breakdown with the relative release of myelin protein fragments [75]. At the same time, the persistence of MBP citrullination in PAD2-deficient conditions highlights the contribution of additional PAD isoforms and underscores the complexity of this regulatory network. However, as anticipated in section 3.3, citrullination does not occur in isolation and is not totally dependent on PAD2. Indeed PAD2-knockout mice showed that citrullination of MBP persists in the absence of PAD2, underscoring the complexity of PAD activity in maintaining myelin stability [41]. As mentioned in section 3.3, the dynamic process of citrullination is highly intertwined with other PTMs [18,49] like arginine methylation that may limit citrullination at specific residues. Since PAD enzymes cannot convert methylated arginine residues to citrulline, arginine methylation may protect MBP from citrullination (Fig. 4) [49]. Given the protective role of methylation and its irreversibility, the interplay with PTMs suggests that the net effect of citrullination depends on a broader molecular context rather than on a single modification event. From a pathological perspective, increased MBP citrullination observed in MS tissue may therefore reflect not only a driver of myelin instability but also an adaptive or compensatory response within a dysregulated system. This perspective is consistent with the concept of heterogeneity of MS lesions and disease stages, where the balance between degeneration, repair, and immune activity is continuously evolving. These observations highlight that MBP citrullination should be interpreted within a spatially and temporally regulated molecular environment, rather than as an isolated pathogenic event. Such complexity also highlights the need for therapeutic strategies that target not only individual pathways but the broader regulatory networks governing myelin homeostasis.

## 5. Is citrullination the proximal event in multiple sclerosis pathogenesis?

The hypothesis that citrullination may act as the proximal chemical change driving MS pathology has attracted widespread attention. Evidence supporting this hypothesis includes early and persistent MBP citrullination, which in experimental models precedes immune response activation, eliciting an inflammatory response and enhancing the secretion of proinflammatory cytokines via activation of nuclear factor kappa B (NF-κB) in astrocytes. Further, up-regulated expression of PAD2 and citMBP observed in NAWM strengthens the hypothesis that citrullination may act as a potential early 'chemical trigger', although this remains unresolved in human MS. Conversely, counterarguments highlight that PAD2 expression is not always associated with MS lesions, with observations reporting reduced PAD2 mRNA levels in NAWM compared to lesion areas. While possible interpretations of these conflicting results have been proposed above, it is also worth noting that MBP hypercitrullination and PAD2 activity may be indirectly triggered before demyelination by the activity of other $Ca^{2+}$-dependent enzymatic processes. A promising candidate can be the activation of a cytosolic $Ca^{2+}$-dependent phospholipase-2 (cPLA2) in OLs that reduces the amount of $Ca^{2+}$ needed to substantially activate PAD2, converting apolar lipids like phosphatidylcholine (PC) into the more lysoPC (LPC). Interestingly, the external application of LPC in mice can increase subtle changes in myelin stability, and studies by Luchicchi et al. showed that LPC levels are significantly higher in prelesional phases of MS WM [5,23]. Moreover, citrullination can be part of normal myelin turnover and OPCs differentiation [63], suggesting that hyper-citrullination may represent a failed repair response rather than a primary cause. The relative contribution of interplay between PAD2 and citrullination, and other PTMs (e.g., arginine, acetylation, and methylation) remains incompletely understood, and it is unclear whether citrullination alone is sufficient to induce or not the MS-associated autoimmunity.

## 6. Future research direction and therapeutic perspective

In light of all these findings, the progressive understanding of MBP citrullination provides a conceptual framework for exploring new pharmacological strategies aimed at preserving myelin integrity in demyelinating disorders. While mapping the spatiotemporal dynamics of citrullination of MBP in early MS stages remains essential, future efforts should prioritize identifying compounds that can target specific components of this biochemical network associated with the molecular mechanisms and main signaling pathways driving the development and progression of MS in a controlled and stage-specific manner. Early-stage interventions could focus on limiting excessive PAD activity associated with increased citrullination to improve clinical symptoms and preserved myelin, whereas later-stage strategies may require immunomodulatory approaches targeting established and compartmentalized autoimmune responses (Fig. 5).

Over the past 20 years, there have been numerous efforts to develop compounds that directly or indirectly inhibit PADs. Although guanidines such as Cl-amidine, Fl-amidine, and their congeners are widely used in preclinical research, they have limitations regarding their pharmacokinetic and pharmacodynamic profiles, which makes them difficult to translate into human clinical use [42,102,103]. In this context, several drug repurposing studies have been conducted. Compounds such as paclitaxel, streptomycin, and various tetracyclines have demonstrated reversible inhibitory properties toward PADs; however, their potency and selectivity are relatively limited [104,105]. Selective PAD2 inhibitors, allosteric modulators, or monoclonal antibodies targeting citMBP epitopes are tangible candidates, but require rigorous pharmacodynamic validation and CNS-specific delivery strategies [106]. Future research should also focus on defining the temporal sequence of citrullination-driven alterations, validating citrullinated MBP as an indicator of early MS pathology. Integrating these tools with advanced imaging and proteomic analyses should not only position MBP citrullination as a clinically useful biomarker for MS patient stratification and guiding stage-specific interventions, but also develop "citrullinome" database corresponding to MS-related pathways, by identifying key target genes and biological pathways in MS brains and the development of novel selective PAD2 inhibitors or metabolic modulators to restore myelin integrity.

## 7. Outstanding questions and future directions

Future research should aim to address outstanding questions to integrate citrullination into a broader metabolic–structural–immune framework, linking $Ca^{2+}$ dysregulation, lipid metabolism, and immune activation (Fig. 4). The application of spatially resolved and single-cell technologies will be instrumental in capturing the heterogeneity of MS lesions and defining the cell-specific dynamics of citrullination across disease stages. Addressing questions such as: Does citrullination precede inflammation in early human MS, or is it a consequence? Can non-invasive imaging or CSF biomarkers detect pre-lesional citrullination? How can PAD2 be selectively inhibited without impairing physiological myelination? and What is the role of other PTMs in modulating

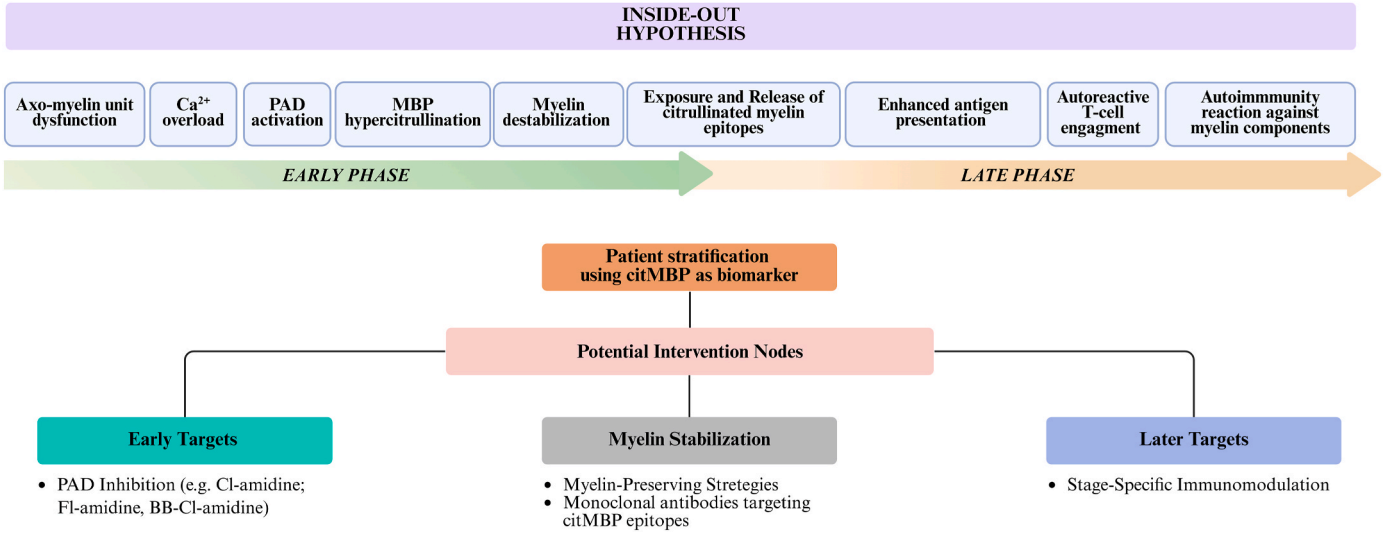


**Fig. 5. Inside-out hypothesis and therapeutic intervention framework in multiple sclerosis.** Schematic representation of the proposed *inside-out* hypothesis of MS, in which early axon-myelin unit dysfunction and $Ca^{2+}$ overload promote PAD activation and MBP citrullination, leading to myelin destabilization. Exposure of citrullinated epitopes may enhance antigen presentation and autoreactive T-cell activation, contributing to inflammatory amplification in later stages. Potential intervention nodes are highlighted along the cascade. Early strategies aim to limit PAD activity, whereas later approaches focus on immunomodulation. CitMBP is also proposed as a candidate biomarker for patient stratification and stage-specific therapeutic targeting.
**Abbreviations:** MS, multiple sclerosis; MBP, myelin basic protein; citMBP, citrullinated MBP; PAD, peptidylarginine deaminase.

citrullination susceptibility? will not only clarify the role of citrullination in MS pathogenesis but also drive the development of mechanism-based, stage-specific therapeutic strategies.

## 8. Concluding remarks

Citrullination has emerged as one of the most relevant and mechanistically compelling PTMs involved in the early phases of myelin destabilization in MS. Accumulating evidence suggests that excessive citrullination of MBP reduces its cationic charge, decreases its ability to compact myelin membranes, and predisposes the sheath to structural fragility. This biochemical vulnerability is detectable before overt inflammation and may therefore represent a potential early contributing step in the transition from pre-immune myelin injury to full autoimmune demyelination. Experimental findings from human tissue, animal models, and cell-based studies converge on the idea that dysregulated PAD enzyme activity, particularly PAD2 in OLs, constitutes a key driver of this pathological process. Importantly, citrullination not only compromises myelin architecture but also generates neo-epitopes capable of amplifying immune recognition, thereby linking early metabolic and structural disturbances to the subsequent autoimmune cascade. Taken together, these data support the hypothesis that aberrant citrullination may represent an early biochemical contributor that activates biochemical pathways of myelin damage and contributes to MS susceptibility and progression. Targeting the molecular pathways that regulate PAD activity and MBP citrullination may open new therapeutic avenues aimed at stabilizing myelin, preventing early degeneration, and delaying the onset or worsening of disease.

## CRediT authorship contribution statement

**Nicola Salvatore Orefice:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing. **Niels R.C. Meijns:** Writing – original draft, Writing – review & editing. **Emanuele Di Martino:** Writing – original draft, Writing – review & editing. **Melissa Schepers:** Writing – original draft, Writing – review & editing. **Assia Tiane:** Writing – original draft, Writing – review & editing. **Geert J. Schenk:** Writing – original draft, Writing – review & editing. **Hugh Kearney:** Writing – original draft, Writing – review & editing. **Tim Vanmierlo:** Writing – original draft, Writing – review & editing. **Antonio Luchicchi:** Conceptualization, Writing – original draft, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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