

# Citrullination: A Target for Disease Intervention in Multiple Sclerosis and other Inflammatory Diseases?

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## Abstract

Citrullinated histone epitopes are involved in the very early stages of inflammatory responses. An important early event is the activation of neutrophils. It has been shown that Peptidyl Arginine Deiminase (PAD) expression levels increase upon pro-inflammatory signalling followed by activation of neutrophils. Subsequently, PAD enzymes cause histone citrullination in the activated neutrophils. Histone citrullination is involved in various processes. One of the most important is NETosis, which results in the release of citrullinated histones to the extracellular space. There, they are involved in Neutrophil Extracellular Trap (NET) formation, which intensifies the inflammatory response. The central role of citrullinated histones in early inflammation makes NETs an attractive target for inflammatory disease intervention. Moreover, the safety profile is expected to be superior to immune-suppressing biologicals, like anti-Tumour Necrosis Factor (TNF) drugs. It is anticipated that shielding citrullinated histone epitopes from the immune system, as well as interfering with their putative roles in the inflammatory response, will have a broad applicability in preventing and treating various inflammatory diseases, including multiple sclerosis.

**Keywords:** Citrullination; Inflammatory diseases; Inflammation; Peptidyl arginine deiminase; Anti-citrullinated protein antibody; Multiple sclerosis; Rheumatoid arthritis

**Abbreviations:** ACPA: Anti-Citrullinated Protein Antibody; AD: Alzheimer's Disease; CK: CytoKeratin; CNS: Central Nervous System; COPD: Chronic Obstructive Pulmonary Disease; GFAP: Glial Fibrillary Acidic Protein; IBD: Inflammatory Bowel Disease; MBP: Myelin Basic Protein; MQ-ACPA: ModiQuest-ACPA; MS: Multiple Sclerosis; NET: Neutrophil Extracellular Trap; PAD: PeptidylArginine Deiminase; PTM: Post-Translational Modification; RA: Rheumatoid Arthritis; SE: Shared Epitope; SLE: Systemic Lupus Erythematosus; TNF: Tumor Necrosis Factor

## Introduction

Protein citrullination, a post-translational modification (PTM) of peptidylarginine, plays an important role in the normal functioning of the immune system, and in physiological processes such as skin keratinization, the insulation of neuronal axons, the plasticity of the central nervous system (CNS), and in gene regulation [1]. Recently, citrullination has become an area of significant interest, because of its suspected role in various pathological conditions, such as rheumatoid arthritis (RA), multiple sclerosis (MS), psoriasis, chronic obstructive pulmonary disease (COPD), and neurodegenerative diseases like Alzheimer's disease (AD) [2]. Moreover, citrullination plays an important role in human and animal cancers through its role in gene regulation [1,3,4]. It is unclear whether citrullination is the cause or the consequence of these pathological disorders [1]. In recent overviews by Baka et al. [1] and Mohanan et al. [4], important aspects of citrullination under both physiological and pathological conditions are discussed. Here, we discuss various aspects of citrullination and the possibility to target the citrullination process as a therapeutic approach to treat inflammatory (autoimmune) diseases.

## The basics of citrullination

Citrullination is a PTM that is catalysed by peptidylarginine deiminases (PADs). PADs are calcium-dependent enzymes that convert

peptidylarginine into peptidylcitrulline [5]. During this reaction, the targeted protein loses a positive charge (Figure 1A), and resulting conformational changes may change the binding properties, promote unfolding, and alter the function and half-life of the protein (Figure 1B).

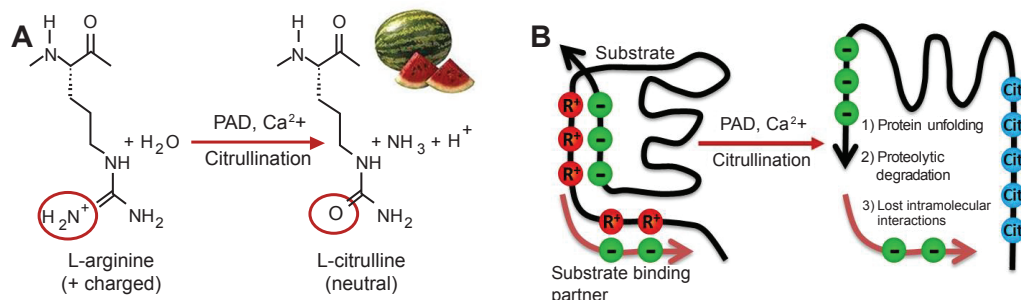
Five PAD isoforms are distinguished, which have different tissue-specific expression patterns. PAD1 is expressed in the epidermis and uterus, although RT-PCR and EST data suggest a broader tissue distribution [6]. PAD2 is the most widely expressed type of PAD. The most abundant expression is observed in skeletal muscle, spleen, secretory glands, and the central nervous system [5]. Citrullination by PAD2 is proposed to play a role in the pathogenesis of MS and AD [2]. PAD3 shows co-expression and co-localization with its natural substrate, trichohyalin, which is a major structural protein of the inner root sheath cells of hair follicles [7]. PAD4 is mainly expressed by cells of the hematopoietic lineage and can therefore be detected in a variety of tissues [5]. It is the only type of PAD that resides in the cell nucleus [8]. PAD4-mediated citrullination of histones may participate in the altered gene regulation in tumourigenesis [9], and is required for the generation of neutrophil extracellular traps (NETs), which is a common feature of the innate immune response to bacteria and fungi. NETs are formed early in inflammatory processes [10] and have been linked to several inflammatory diseases [11] and to various human autoimmune disorders, including systemic lupus erythematosus (SLE) [12]. Increased PAD2 and PAD4 expression, accompanied by the production

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**Figure 1: The process and the effects of citrullination.**

Watermelon (*Citrullus lanatus* L.) contains abundant arginine and citrulline as free amino acids. **A)** Peptidylarginine deiminase (PAD) enzymes convert peptidylarginine to peptidylcitrulline in a process called citrullination. The difference between arginine and citrulline is depicted in the red circles. **B)** The effects of PAD-catalysed citrullination. Loss of ionic interactions due to citrullination may destabilize intra- and intermolecular interactions, leading to the disruption of protein complexes and/or protein unfolding.

of anti-citrullinated protein antibodies (ACPAs) has been observed in the inflamed synovium of RA patients [13]. PAD6 is found in early embryos and ovaries [5,14], and has been less intensively investigated than PAD2 or PAD4. To date, only a single prokaryotic enzyme that can citrullinate proteins has been identified in *Porphyromonas gingivalis*. This enzyme (AAF06719), however, is not evolutionarily related to the vertebrate PAD enzymes, can convert both peptidylarginine and free L-arginine, is not dependent on  $Ca^{2+}$ , and shares sequence homology with several arginine deiminases [15]. Recently, a possible connection between *P. gingivalis* and RA was proposed [16].

### Citrullination in normal physiology

**Skin keratinization:** In the skin, proteins are altered by cleavage, cross-linking, and by amino acid side chain modifications (e.g. citrullination) that help to create a matrix, which is resistant to insults (Table 1). To date, three molecules are known to be citrullinated in the epidermis: (pro)filaggrin and keratins K1 and K10. Cytokeratins (CKs) constitute the main intermediate filament produced by keratinocytes building up keratin filaments in the skin, hair, and nail. Citrullination enables these proteins to bind to each other by changing the charge of the interacting surfaces. Native CK and loricrin have very low affinity for each other, since both proteins are very basic [17]. Citrullinated CK on the other hand, binds well to loricrin and possibly also to desmoplakin. Desmoplakin is a desmosomal protein that helps the keratin matrix to extend transcellularly [40]. CK citrullination has also been implicated in the pathogenesis of psoriasis (as discussed later in this review).

Citrullination of profilaggrin facilitates its cleavage by proteases into smaller filaggrin units [17]. The modified filaggrin molecules can bundle keratins into a three-dimensional structure.

**Myelin formation in the central nervous system (CNS):** In the CNS, PAD2 is mainly expressed by oligodendrocytes, astrocytes and microglia. The enzyme citrullinates myelin basic protein (MBP), glial fibrillary acidic protein (GFAP), and other proteins [17]. Citrullination of MBP was suggested to be essential for the plasticity of the CNS at young age, since the level of MBP citrullination changes rapidly postnatally [17] (Table 1).

**Gene regulation:** Gene regulation is the major focus of epigenetic research. It is fine-tuned by PTMs of histones and coordinated by counteracting enzymes such as histone acetyltransferases, histone deacetylases, methyltransferases, demethylases, and PADs (Figure 2).

In contrast to other PADs, only PAD4 has been proposed to localize to nuclei and contains a putative nuclear localization signal, which is consistent with a role in gene regulation [5]. PAD4 can citrullinate arginines and methylarginines of histone 3 and histone 4 and, as a consequence, prevents or reverses methylation of these histone residues [41]. Citrullination may be one of the crucial epigenetic regulatory mechanisms (Table 1 and Figure 2).

**Immune functions:** The cells of the hematopoietic lineage (especially monocytes and granulocytes) express PAD4, suggesting that citrullination has a key role in the physiology of these cells. One of the mechanisms by which neutrophils trap and kill bacteria is by forming highly decondensed chromatin structures, termed NETs [42] (Table 1). Histone hypercitrullination catalysed by PAD4 is essential for this process, since PAD4 knockout mice cannot form NETs after stimulation with chemokines or incubation with bacteria, and are deficient in bacterial killing by NETs [26].

Results obtained from studies with macrophage cell lines suggest that PAD2 interacts with  $\kappa B$  kinase, and suppresses NF- $\kappa B$  activity after lipopolysaccharide stimulation, indicating the involvement of PAD2 in innate immune defense [27]. Furthermore, naturally occurring citrullinated chemokines have been shown to be less potent than the arginine-containing variants [28] (Table 1). Citrullinated CXCL8 (IL-8) has reduced affinity for heparin, is resistant to thrombin- or plasmin-dependent cleavage into a more potent CXCL8 fragment, and is unable to attract neutrophils to the peritoneum. In contrast, citrullination of CXCL8 significantly increases the chemokine's ability to recruit neutrophils from the bone marrow into the blood circulation, and impairs its clearance from the circulation, thereby maintaining serum leukocyte levels [29]. Modification of CXCL12 by one citrulline severely impairs CXCR4 binding and signalling (calcium mobilization, phosphorylation of ERK and protein kinase B), while maximally citrullinated CXCL12 is inactive [30]. Citrullination also reduces the chemoattractive and signalling capacity of CXCL10 and CXCL11 on CXCR3, and impairs T cell activation. Cytokines may influence PAD activity as well. For example, TNF treatment induces the translocation of PAD4 from the cytosol to the nucleus in oligodendroglial cell lines [31]. Transgenic mice overexpressing TNF have increased levels of citrullinated histones and nuclear PAD4. These examples illustrate the versatility of PADs with regard to predominantly anti-inflammatory and antibacterial effects (Table 1).

### Citrullination in normal physiology

#### Epidermis

- Keratinization (keratin, trichohyalin, filaggrin) [17]

#### Nervous system

- Myelin sheath stability
- Plasticity of the brain [17]

#### Gene regulation

- P53 pathway [18-23]
- Estrogen pathway [24,25]

### Citrullination in pathophysiology

#### Innate immune responses

- PAD4-catalysed histone hypercitrullination is essential in NET formation [26]
- PAD2 interacts with inhibitor  $\kappa$ B kinase, and suppresses NF- $\kappa$ B activity in macrophages after lipopolysaccharide stimulation [27]

#### Chemokines

- Citrullinated CXCL8
  - 1) has reduced affinity to glycosaminoglycans
  - 2) is resistant to thrombin/plasmin-dependent cleavage
  - 3) is unable to attract neutrophils to the peritoneum
  - 4) can more efficiently recruit neutrophils into the blood circulation [28]
- Citrullinated CXCL12 has reduced effects through CXCR4 [29]
- Citrullinated CXCL10 and CXCL11 have decreased chemoattracting and signalling capacity through CXCR3 [30]

#### Effects of cytokines

- TNF induces the translocation of PAD4 to the nucleus [31]

#### Psoriasis

- Hypocitrullination of CK1 [27]

#### Tumorigenesis

- Increased tissue citrullination [9]
- Increased tissue and serum PAD4 [32,33]
- PAD4 interference with p53 pathway [34]
- Citrullination alters AT and CK [9,32,35]

#### Rheumatoid arthritis

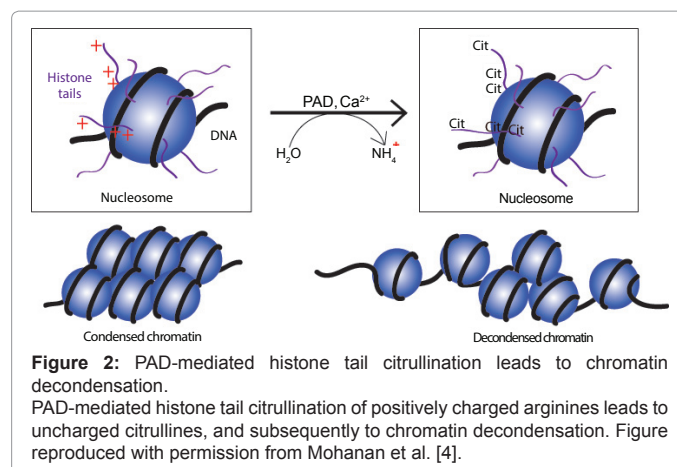
- Triggering of protein citrullination, followed by ACPA generation and disease onset, induced by
  - 1) Genetic factors (*HLA-DRB1*, *PTPN22*) [36,37]
  - 2) Environmental factors (infection, smoking) [16,38]

#### Multiple sclerosis

- Hypercitrullination of MBP [39]

**Table 1:** Roles of PADs and citrullination in (patho) physiology.

**Female reproduction:** PAD6 was found to be mainly expressed in ovary, oocytes, and the early embryo. PAD6 is essential for female fertility and in PAD6-null mice developmental arrest was observed at the two-cell stage. In addition, PAD6 is essential for the formation of a cytoskeletal structure (termed lattices) in oocytes and early embryos. Given the abundance of PAD 1-4 in the female reproductive tissue and the regulation of their expression by estrogen [2], it seems very likely that, in addition to PAD6, the other PAD enzymes play important roles in the female reproductive system as well.



**Other functions:** PAD expression has been detected in other tissues as well. For example, PAD1 is also expressed in uterus. In addition to the CNS, PAD2 is expressed in various other tissues, including skeletal muscle, uterus, salivary glands, pancreas, bone marrow, macrophages, and epidermis. PAD3 has been detected in hair follicles and epidermis, while PAD6 expression was detected in ovary, peripheral blood leukocytes, and testis. The activities and functions of PADs in these tissues largely remain to be elucidated.

### Citrullination and disease

The first disease for which a close linkage between citrullination and pathophysiology was established is RA. RA is a multifactorial disease in which genetic and environmental factors play an important role. Specific HLA-alleles, more precisely HLA-DRB1 alleles encoding the shared epitope (SE), result in RA-risk motifs on the HLA molecule [36]. Another example of a significant genetic predisposition is a polymorphism of PTPN22 that increases RA-risk in SE carriers [37]. Various environmental factors are suggested to influence disease development, the best documented of which is smoking [38].

Citrullinated proteins present in the joints are supposed to be a factor that causes ongoing inflammation in RA [43,44]. Moreover, anti-citrullinated protein antibodies (ACPAs) are a diagnostic marker for RA, which can be found long before the onset of the disease [45].

Recently, we generated a subset of human recombinant ACPAs, termed ModiQuest-ACPAs (MQ-ACPAs), which prevent the onset of arthritis in both collagen-induced arthritis and collagen-antibody-induced arthritis mouse models [46]. Therapeutic administration of these antibodies results in the arrest of joint inflammation and prevents a further increase of the inflammatory response. The differentiating epitope that is recognized by MQ-ACPAs is the citrullinated N-terminus of histone 2A [46]. Next to RA, citrullination has been linked to various other diseases such as:

**Periodontitis:** Periodontitis is a chronic progressive inflammation that leads to bone resorption in the oral cavity. The major causative agent of the disease is *P. gingivalis*, especially in genetically predisposed individuals (HLA DRB1\*04 alleles). Wegner et al. reported that *P. gingivalis* PAD rapidly citrullinates both bacterial and host peptides (fibrinogen and  $\alpha$ -enolase) [47]. The citrullinated immuno-dominant epitope region (CEP-1) is 100% identical to an epitope present in *P. gingivalis* enolase, and antibodies to CEP-1 (found in 40–60% of RA patients) react with both human and *P. gingivalis* enolase [48]. Moreover, citrullinated  $\alpha$ -enolase is found in RA synovial fluid [49],

suggesting molecular mimicry (enolase) in the pathogenesis of RA.

**Psoriasis:** Psoriasis is a chronic immune-mediated disease, characterized by red scaly plaques on the skin, which sometimes is accompanied by arthritis. In psoriasis, keratinocytes proliferate very rapidly and travel from the basal layer to the surface in only about four days. This process normally takes about a month. The skin cannot shed these cells quickly enough so they accumulate in thick, dry patches or plaques. In normal keratinocytes, keratin 1 (CK1) is citrullinated by PAD1 during terminal differentiation. This process causes the keratin filaments to become more compact, which is essential for the normal cornification process of the epidermis. The keratinocytes in the psoriatic hyperproliferative plaques do not contain citrullinated CK1 [17] (Table 1). It is intriguing that a small percentage of psoriasis patients with arthritis have ACPA, however, the presence of ACPA did not relate to radiological changes and/or deformity and functional impairment [50].

**Inflammatory bowel disease (IBD):** IBD, mainly Crohn's disease and ulcerative colitis, is a dynamic, chronic inflammatory condition that is associated with an increased risk of colon cancer. Inflammatory cell apoptosis is a key mechanism for regulating IBD. PAD levels, as well as the levels of citrullinated proteins are elevated in mouse and human colitis [51,52]. Recently, Chumanevich et al. showed that the inhibition of citrullination results in a decreased inflammation in IBD [52].

**Systemic lupus erythematosus (SLE):** SLE is a systemic autoimmune disease that can affect any part of the body. As in other autoimmune diseases, the immune system attacks the body's cells and tissues, resulting in inflammation and tissue damage. Inflamed tissues of SLE patients show increased levels of citrullinated proteins, including NET-associated histones [9]. Citrullination of histones arising from PAD4 activity during NETosis was recently shown to be a specific marker of NETs and necessary for NET formation [41,53]. Specific PTMs of histones within NETs may drive the process by which tolerance to NET-associated proteins is broken.

**Alzheimer's disease (AD):** Abnormal accumulation of citrullinated proteins, such as GFAP, is found in the hippocampus of AD brains and these modified proteins show increased immunoreactivity, compared to proteins from normal brain. Recently, selective expression of PAD2 and PAD4 in astrocytes and neurons was reported [54]. Citrullination of cerebral proteins by PAD2 occurs in regions undergoing neurodegeneration, suggesting that citrullination may promote the progression of neurodegenerative diseases [55,56].

**Multiple sclerosis (MS):** MS is an autoimmune demyelinating disease of the CNS with multifactorial etiology. Demyelination causes loss of nerve signals, which in turn results in many clinical manifestations (e.g. visual loss, extra-ocular movement disorders, paresthesias, loss of sensation, weakness, dysarthria, spasticity, ataxia, and bladder dysfunction). The development of MS might involve epigenetic changes, which are passed from parent to offspring, and are highly sensitive to environmental influences, such as smoking or vitamin D deficiency. These epigenetic mechanisms include DNA methylation, histone modification and microRNA-associated post-transcriptional gene silencing [57]. Moscarello et al. proposed that myelin damage in MS white matter results from a failure to maintain the myelin sheath, due to enhanced citrullination of MBP [39] (Table 1). In approximately 18% of the MBP molecules of healthy adult humans, 6 out of 19 arginines are citrullinated (MBP-cit<sub>6</sub>: R<sub>25</sub>, R<sub>31</sub>, R<sub>122</sub>, R<sub>130</sub>, R<sub>159</sub>, R<sub>267</sub>). The remaining MBP molecules do not contain citrulline. In MS patients, the proportion of MBP-cit<sub>6</sub> is increased to 45% of total MBP [58]. The decreased net positive charge of MBP-cit<sub>6</sub> causes partial unfolding of

MBP molecules resulting in a more open conformation. Although MBP-cit<sub>6</sub> is capable of forming lipid complexes more rapidly than non-citrullinated MBP, these hypercitrullinated proteins are unable to organize lipid bilayers into compact multilayers, which in turn lead to instability of the myelin sheath [59]. The stability of the myelin sheath is further compromised due to an increased susceptibility of MBP-cit<sub>6</sub> to proteases such as cathepsin D [60]. The cause of hypercitrullination may be increased PAD2 and PAD4 expression levels [31] and/or hypomethylation of the PAD2 promoter [61]. In PAD2-knockout mice, CNS citrullination is abolished and demyelination is not seen [62]. Also, protein-hypercitrullination is reversed by inhibition of PAD2 in autoimmune MS mouse models [63]. Modulation of the activity of PAD4 by means of small molecule inhibitors indicates the potential therapeutic use for PAD inhibitors in MS pathology [64]. In a transgenic mouse line containing multiple copies of PAD2 cDNA, increased severity of clinical symptoms of MS is observed, in line with increased PAD2 expression and MBP citrullination [65]. Citrullinated peptide fragments from MBP elicit a Th1-polarized response of T cells isolated from MS patients [66]. Recently, PAD2 was shown to be located in the nucleus of mammary epithelial cells and in neuronal cells. Like PAD4, PAD2 may citrullinate histones [67,68], and therefore may play a role in gene regulation. In a transgenic mouse model overexpressing TNF, an elevated nuclear presence of PAD4 as well as increased levels of citrullinated histones were detected in the CNS prior to demyelination [31]. Taken together, increasing evidence indicates that (early) inflammatory responses play a role in the onset and progression of neurodegenerative diseases, though a causative role for citrullination in these conditions remains to be established.

### Citrullination plays a fundamental role in inflammatory events

Historically, PAD activity is strongly associated with the regulation of autoimmune-mediated inflammatory events. The presence of citrullinated proteins prior to the onset of RA [69] indicates the contribution of PAD-mediated citrullination in autoimmunity. In MS patients, both PAD2 and PAD4 are significantly upregulated if compared to healthy individuals, possibly contributing to the development of the autoimmune responses in MS [70].

Recent studies suggest that PAD-mediated citrullination is elevated in a variety of inflammatory diseases, which lack a strong autoimmune component, such as COPD and chronic tonsillitis [51,71,72]. Perhaps the best demonstration that PAD-mediated citrullination can facilitate non-autoimmune inflammatory events is the recent finding that PAD activity is strongly up-regulated in inflamed tissue, following a sterile skin punch biopsy procedure in mice [73]. Thus, it can be inferred that citrullination plays a critical and fundamental role in inflammatory events induced by a range of pathologies, both infectious and non-infectious.

It has become clear that citrullination is not a particular disease-related event, but an inflammation-dependent process occurring in various inflamed tissues [51], like MS, RA, osteoarthritis, psoriatic arthritis, juvenile idiopathic arthritis, spondyloarthropathy, Parkinson's disease, psoriasis, AD, autoimmune hepatitis, Lewy body dementia, and multiple system atrophy.

PAD4, expressed by infiltrating immune cells, plays an important role in (early) inflammatory processes. The activation of PAD4 and the subsequent citrullination of histones are involved in the inflammatory process. Citrullination of histones might result in the exposure of

neo-epitopes to the immune system, and aid to the many processes in which citrullinated histones play a role, such as the formation and stabilisation of NETs [74]. In addition, citrullination may be involved in anchoring various molecules to NETs, such as immune response stimulators (LL37, HMGB-1, elastase, and myeloperoxidase), and may thus promote the recruitment of phagocytes and other inflammatory immune cells. Finally, different histone modifications in NETs may activate or dampen the inflammatory response by acting on innate pattern recognition receptors [10].

### **Interfering with citrullination as a novel therapeutic approach for inflammatory diseases**

Recently, various studies have shown that interfering with citrullination diminishes inflammation under various inflammatory conditions [45,52,75,76], and may be applicable in cancer therapy [34].

There are some obvious approaches to interfere with citrullination and its various roles in inflammation. One approach would be the use of PAD inhibitors [64,77-81]. It will be clear from the complex and diverse processes mediated by PAD enzymes that interfering with the activity of these enzymes provides serious challenges in regard to the specificity and potential toxicity of such inhibitors. PAD inhibition might induce severe adverse effects in skin physiology, development of CNS, gene regulation, the function of immune system, the female reproductive system, etc.

Targeting of PAD inhibitors to specific cells or tissues may reduce the risk of negative side effects and toxicity of these compounds. For instance, inhibiting PAD activity in tumour cells, via tumour cell-specific internalizing antibodies, might be a means to cause cytotoxic effects specifically in these tumour cells. Systemic application of PAD inhibitors requires a favorable balance between health benefit obtained and negative side effects introduced. This problem will be more pronounced when non-isoform-specific PAD inhibitors are used.

An alternative approach, avoiding the above-mentioned pitfalls, is the use of antibodies to block specific citrullinated epitopes that play a critical role in the inflammatory process.

As described above, various studies show that PAD enzyme expression is elevated upon initial inflammatory stimuli. This may cause (aberrant) citrullination of target molecules, which subsequently leads to pathogenic effects. In RA, increased PAD levels in the vicinity of citrullinated fibrin deposits are thought to be responsible for, and contribute to, the exacerbation of the disease [82]. PAD expression in neutrophils increases upon pro-inflammatory signalling, activating these cells. Subsequently, in activated neutrophils, PAD enzymes cause histone citrullination during NETosis, as described above [83]. The central role of citrullinated histones in early inflammatory responses makes it an attractive novel target for inflammatory disease intervention [46,84]. Indeed, specific citrullinated histone epitopes appear to be the differentiating epitopes for interference with the inflammatory response in animal models for arthritis using citrullinated histone-specific antibodies (MQ-ACPAs) [46].

MQ-ACPAs were identified by screening recombinant human antibody phage display libraries made from antibody-producing cells obtained from RA patients. This yielded a subset of ACPA molecules recognizing a specific citrullinated peptide, which were subsequently tested in an arthritis animal model for their capacity to prevent the onset of inflammation and avoid joint damage. Only a very limited subset of antibodies showed a therapeutic effect. In a reiterative process of testing

and optimization (including humanization), a number of antibody candidates were generated and their epitope target, a citrullinated histone, was identified. Some of these recombinant monoclonal ACPAs were tested for efficacy in two RA mouse models and the MQ-ACPA with the highest efficacy in the animal models is currently in preclinical development [46].

Mice studies have shown that this innovative approach prevents the onset of the inflammatory response and the progression of the inflammation. Also, these animal studies showed that during full-blown arthritis, combination treatment with steroids and MQ-ACPA reduces joint swelling and subsequent flares, and further prevents joint damage to near normal levels without detectable side effects [46]. It is anticipated that in patients, after a limited period of treatment with MQ-ACPA, the immune system activation will have decreased to normal levels. Interference at an early stage will result in long-term remission and provide considerable cost benefits over current long-term treatment regimens. For patients this means fewer side effects, no further joint damage, less pain, and a significantly improved quality of life.

### **Future anti-inflammatory therapeutics**

As reviewed in this manuscript, citrullination of proteins has many physiological functions. Aberrant citrullination however, can play a role in the pathology of various diseases. Citrullinated histone epitopes are involved in the onset of the inflammatory responses.

Recently, various studies have shown that interfering with protein citrullination results in the attenuation of symptoms in several inflammatory disease animal models. In mouse models for IBD and RA it was demonstrated that the inhibition of PAD enzymes reduces inflammation [16,48-50].

Moscarello et al. recently reported that the amount of PAD enzymes and protein-citrullination in normal-appearing white matter of MS patients is enhanced, as compared to healthy individuals [63]. Protein-hypercitrullination in neurodegenerative as well as autoimmune MS mouse models strongly correlates with disease severity and progression. Treatment with PAD inhibitors decreases PAD enzyme activity in the brain and in the spinal cord of these mice, resulting in lower amounts of citrullinated proteins and in remyelination of the CNS [63].

Inhibition of the PAD enzymes that are responsible for citrullination is a rational approach by which one can interfere with the protein citrullination pathway and thus with its various roles in inflammation [49,50]. Specific targeting of PAD inhibitors to cells or tissues may reduce the risk of negative side effects and cytotoxicity of these compounds. An alternative, more focussed approach, avoiding potential side effects and toxicity of PAD inhibitors, is the use of antibodies to block specific citrullinated epitopes that play critical roles in the inflammatory process.

Currently, the therapeutic needs in MS are largely unmet. Most therapies are based on modulation of a particular aspect of the immune system, without addressing the underlying causative process. Although some improvement in relapse rates has been observed, the basic causative pathology continues to exist.

Here, we propose that interfering with PAD enzyme activity and/or citrullination may serve as a novel treatment strategy to enhance the MS patients' quality of life.

The efficacy of antibodies (MQ-ACPAs) to block specific citrullinated epitopes that play critical roles in the inflammatory process was recently demonstrated in arthritis mouse models. MQ-ACPAs block a specific citrullinated histone epitope that is only present during inflammatory processes, resulting in the prevention of inflammation onset in RA animal models [46].

We anticipate that MQ-ACPAs, and other compounds that shield citrullinated epitopes from the immune system, will have a broad applicability in the prevention and treatment of various inflammatory diseases, such as MS and RA.

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