Clinic Rev Allerg Immunol (2015) 49:232–239
DOI 10.1007/s12016-014-8459-2

Citrullinated Autoantigens: From Diagnostic Markers to Pathogenetic Mechanisms

Sylviane Muller · Marko Radic

Published online: 30 October 2014

Abstract The conversion of an arginine residue in a protein to a citrulline residue, a reaction carried out by enzymes called peptidylarginine deiminases (PADs), is rather subtle. One of the terminal imide groups in arginine is replaced by oxygen in citrulline, thus resulting in the loss of positive charge and the gain of 1 dalton. This post-translational modification by PAD enzymes is conserved in vertebrates and affects specific substrates during development and in various mature cell lineages. Citrullination offers a unique perspective on autoimmunity because PAD activity is stringently regulated, yet autoantibodies to citrullinated proteins predictably arise. Autoantigens recognized by anti-citrullinated protein antibodies (ACPA) include extracellular proteins such as filaggrin, collagen II, fibrinogen, and calreticulin; membrane-associated proteins such as myelin basic protein; cytoplasmic proteins such as vimentin and enolase; and even nuclear proteins such as histones. Some ACPA are remarkably effective as diagnostics in autoimmune disorders, most notably rheumatoid arthritis (RA). Several ACPA can be observed before other clinical RA manifestations are apparent. In patients with RA, ACPA may attain a sensitivity that exceeds 70 % and specificity that approaches 96–98 %. The biological context that may account for the induction of ACPA emerges from studies of the cellular response of the innate immune system to acute or chronic stimuli. In response to infections or inflammation, neutrophil granulocytes activate PAD, citrullinate multiple autoantigens, and expel chromatin from the cell. The externalized chromatin is called a neutrophil extracellular "trap" (NET). Citrullination of core and linker histones occurs prior to the release of chromatin from neutrophils, thus implicating the regulation of citrullinated chromatin release in the development of autoreactivity. The citrullination of extracellular autoantigens likely follows the release of NETs and associated PADs. Autoantibodies to citrullinated histones arise in RA, systemic lupus erythematosus, and Felty's syndrome patients. The citrullination of linker histone H1 may play a key role in NET release because the H1 histone regulates the entry and exit of DNA from the nucleosome. Juxtaposition of citrullinated histones with infectious pathogens and complement and immune complexes may compromise tolerance of nuclear autoantigens and promote autoimmunity.

Keywords Autoantibodies · Citrulline · Histones · Neutrophil extracellular traps (NETs)

Introduction

The initial discovery of citrulline residues in proteins seemed to be a biochemical anomaly as it contradicted the central dogma of molecular biology. Since there is no codon or tRNA for citrulline, this amino acid residue cannot be translationally incorporated into newly synthesized proteins. However, decades ago, proteins containing citrulline were unambiguously identified in several mammalian tissues [1]. Thus, enzymes that convert arginine residues to citrulline residues in a protein were predicted to exist, and indeed, distinct enzymes were subsequently discovered in vertebrate species ranging from fish to humans and they were successfully purified from skin, muscle, and hair follicles [2, 3]. In total, higher eukaryotes express five peptidyl arginine deiminases (PADs), which modify proteins with important tissue-specific functions.

S. Muller
Immunopathology and Therapeutic Chemistry/Laboratory of Excellence MEDALIS, CNRS, Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France

M. Radic (✉)
Department of Microbiology, Immunology and Biochemistry, University of Tennessee Health Science Center, Memphis, TN, USA
e-mail: mradic@uthsc.edu

However, the significance of this arginine modification remained uncertain until additional substrates were identified.

Among the first deimination substrates that were identified in the epidermis were keratin and filaggrin, two autoantigens targeted in disorders as diverse as pemphigus and rheumatoid arthritis (RA) [4, 5]. These studies identified filaggrin as the target of the previously unexplained reactivity of RA sera with rat epithelia [6]. These results prompted the search for the specific molecular determinants that account for RA reactivity. The search was directed toward a specific sequence contained within filaggrin, a protein that contains a high number of citrulline residues. Positive "hits" were recorded against peptides matching the sequence of filaggrin provided that the peptides incorporated citrulline residues in place of arginine residues during synthesis [7]. Such peptides proved to be particularly useful substrates in assays used to diagnose RA [2, 8–12]. The usefulness of the peptides was increased by joining their ends to form a loop. Such cyclic citrullinated peptides (CCP) were used for the development of an ELISA that attains a high sensitivity and exhibits remarkable specificity for RA over other autoimmune conditions [8]. The assay has since been optimized, and it is included among the revised 2010 classification criteria for RA [13].

Preceding studies on autoimmunity in multiple sclerosis (MS) patients had indicated that myelin basic protein (MBP) exists in alternative isoforms, which contain variable numbers of citrulline residues and differ by their isoelectric points, and that these isoforms differentially react with T cell lines from MS patients [14]. The number of citrulline residues was observed to change during development and during progression of MS [15]. Thus, deimination was shown to be regulated during development and to dictate immunoreactivity of autoantibodies in diverse disorders. These discoveries have had profound impacts on the diagnosis and clinical evaluation of RA and related autoimmune disorders.

Autoantibodies Recognize Citrulline in Diverse Autoantigens

The remarkable success of the anti-CCP assay in the detection of RA led to a sustained, worldwide effort to identify additional citrullinated autoantigens (Table 1). These efforts also shed light on the biological mechanisms that drive the conversion of arginine residues in proteins to citrulline residues. Early on, it was recognized that filaggrin is not the only extracellular matrix protein that is modified by PADs. Other important proteins that are deiminated by PADs and whose citrulline residues form part of the epitopes recognized by RA autoantibodies include collagen type II and fibrinogen. Elevated levels of citrullinated collagen II in the synovium of RA patients [16] suggested that PAD-mediated modification of cartilage in RA joints may directly contribute to the induction of autoantibodies. In parallel, a citrullinated peptide derived from the primary sequence of fibrin (residues 60 to 74) was shown to be useful as a clinical diagnostic for RA with a sensitivity of 74 % and a specificity of 95 % [12].

Interestingly, calreticulin, a plasma protein that is involved in binding to apoptotic cells [17] and that contributes to innate system activation, can recognize the conserved domain of RA-predisposing HLA molecules (the so-called "shared epitope"). Furthermore, the binding is enhanced by deimination of calreticulin [18]. This observation suggests that deimination of certain plasma proteins may play a regulatory role in the clearance of cell remnants. A similar anti-inflammatory role may be ascribed to the deimination of cytokines such as CXCL8, CXCL10, and TNF [19–21]. The deiminated cytokines have a reduced chemotactic potency and a decreased stimulatory effect on other immune cells. Perhaps, autoantibodies to citrullinated autoantigens may also have a beneficial effect by enhancing clearance of the modified antigens.

Conversely, increased citrullination of autoantigens such as myelin basic protein is associated with impaired function of the affected organs [22]. Similarly, the deimination of filaggrin promotes the unfolding and degradation of this structural protein [23], and the deimination of fibronectin decreases its function as ligand for adhesion receptors [24]. Deimination also has destabilizing effects on cytoplasmic proteins. Citrullinated vimentin is found at elevated levels in the synovial fluid and in circulating immune complexes of RA patients [25], and vimentin deimination is linked to the disassembly of the cytoskeleton [26]. By analogy, the deimination of F-actin capping protein presumably deregulates the formation of actin fibers [27].

Three additional proteins are preferentially recognized in their citrullinated form. The citrullinated form of the immunoglobulin chaperone BiP is preferentially bound by RA patients' sera, and treatment of mice with citrullinated BiP promotes experimental arthritis [28]. The deiminated HSP90 heat shock protein was identified as a useful diagnostic autoantigen in interstitial lung disease that is a potentially serious manifestation of RA [29]. The deimination of enolase, a glycolytic enzyme, may play a role during the infection of gingival epithelial cells [30]. This enzyme is one of the substrates of the bacterial PAD, which is expressed by the periodontal pathogen *Porphyromonas gingivalis*. The discovery of infection-induced autoantigen deimination supports the intriguing possibility that an oral pathogen could induce autoantigen modifications and thus break immune tolerance. Clearly, analysis of anti-citrullinated protein autoantibodies (ACPA) has fostered the emergence of productive new areas of research (see [31, 32] as examples).

One immediate obvious implication of autoantibodies to citrullinated epitopes is that the post-translational modification (PTM) itself is the protagonist in converting the autoantigens

Table 1 Examples of autoantigens that acquire citrulline residues

	Function	Reference
Extracellular PAD substrates		
Filaggrin	Outer nucleated layer of epidermis	[7]
Collagen II	Component of joint cartilage	[16]
Fibrinogen/fibrin	Component of blood clots	[12]
Calreticulin	Plasma protein	[18]
Cytokines (CXCL8 and CXCL10)	Immune cell signaling	[73]
Membrane-associated substrate		
Myelin basic protein	Formation of axon sheath	[22]
Cytoplasmic PAD substrates		
Vimentin	Intermediate filaments	[25]
F-actin capping protein	Regulation of actin cytoskeleton	[27]
BiP (heavy chain-binding protein)	Immunoglobulin chaperone	[28]
HSP90	Heat shock protein of 90 kD	[29]
Enolase	Glycolytic enzyme	[30]
Nuclear PAD4 substrates		
Histones H1, H2A, H3, and H4	Structure of chromatin	[34, 59]
Peptidylarginine deiminase 4 (PAD4)	Nuclear deiminase	[68]

into stimuli for the adaptive immune system. Tolerance is a strong force that normally prevents the activation of autoreactive B and T lymphocytes in individuals who remain free of autoimmune disease. Therefore, the central question in autoimmune disease research is to account for the initial events that break tolerance and lead to the specific recognition of autoantigens. One possibility, suggested by the prevalent occurrence of ACPA, was that the conversion of specific arginine residues into citrulline residues alters the recognition of B cell receptors and/or T cell receptors to such a degree that tolerance is evaded and lymphocytes to the modified autoantigens proliferate [33]. However, it remained a matter of speculation what conditions were likely to lead to a drastic change in the amount of citrullinated proteins. Possible candidates for these conditions were discovered in the course of studies into the regulation of PADs.

Histone Deimination

Deeper understanding of autoantigen deimination came from careful analysis of stimuli that causes the activation of PAD4, the only PAD that is localized to the nucleus and abundantly expressed in granulocytes and monocytes [2, 3]. Immunofluorescence with an anti-PAD4 monoclonal antibody demonstrated the highly variable expression levels and heterogeneous cellular distribution of PAD4 in human blood neutrophils (Fig. 1). Because PAD activity strictly depends on calcium, Hagiwara et al. induced granulocyte differentiation in HL-60 cells and then exposed them to calcium ionophore [34]. This treatment raised intracellular calcium levels and induced deimination. Antibodies to modified citrulline were used in two-dimensional protein gel electrophoresis to identify

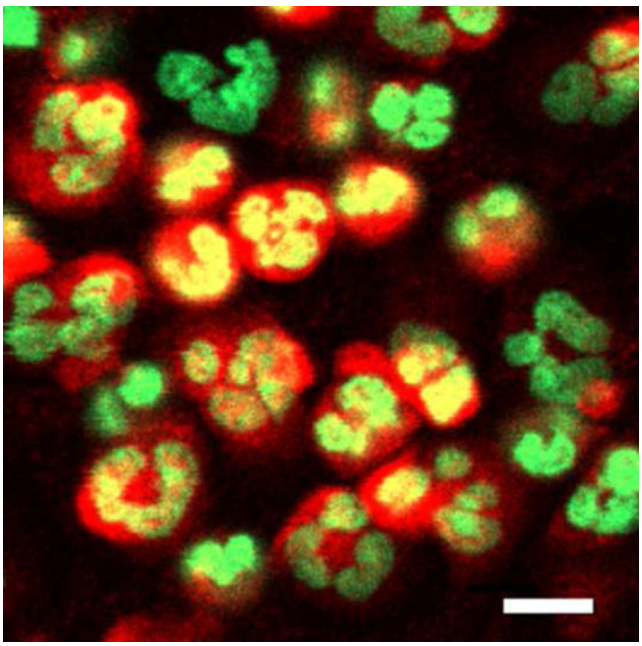

Fig. 1 Detection of peptidylarginine deiminase 4 in human blood neutrophils. Human neutrophils were purified, as described [35], and incubated with a mouse monoclonal antibody to PAD4 (kind gift of Dr. Nakashima, Japan). The antibody was detected with a fluorescent anti-mouse antibody (shown in *red*) and the nuclear DNA was visualized with Sytox *green*. The overlap between the two colors yields *yellow*. All cells in this preparation exhibit the typical lobulated granulocyte nucleus, indicating that cells were highly purified. The PAD4 signal is heterogeneous in different neutrophils, suggesting that PAD4 is localized to nuclei and cytoplasm in these cells. The *bar* indicates 10 μm

nucleophosmin and three of the four core histones (H2A, H3, and H4) as abundant substrates of PAD4 [34]. This and subsequent studies implicated apoptosis in the induction of histone deimination. Because granulocyte apoptosis is induced during the resolution phase of an inflammatory response, it was proposed that increased amounts of citrullinated autoantigens may be generated during an inflammatory response in vivo.

However, the idea that apoptosis induces deimination proved incorrect. This was concluded from studies showing that classic stimuli for apoptosis fail to induce PAD activation, and caspase inhibitors are unable to block deimination [35]. The solution to this dilemma was provided by experiments that identified a new form of neutrophil cell death. Brinkman and Zychlinski discovered that neutrophils initiate a programmed cell death quite distinct from apoptosis when in contact with bacteria, yeast, or viruses [36–38]. This program proceeds through stages of nuclear and granule membrane dissolution, chromatin unwinding, and the release of chromatin from the cell [39]. Because the extracellular chromatin may capture and immobilize microbes, the authors coined the expression "neutrophil extracellular traps" (NETs), and nowadays, this form of cell death is known as NETosis [37, 38].

Neeli et al. were the first to connect histone deimination with NET release by showing that HL-60 granulocytes and primary human blood neutrophils respond to numerous stimuli associated with infections or inflammation by rapidly inducing histone deimination [35]. The stimuli can be as diverse as lipopolysaccharide (LPS), tumor necrosis factor (TNF), hydrogen peroxide, or lipoteichoic acid. The specific induction of histone deimination can be visualized by immunofluorescence with antibodies that react with deiminated histone H3. Neeli et al. used this technique and discovered that deiminated histones are incorporated into NETs [35]. Alternatively, citrulline residues can be visualized with a chemical probe that reacts with the functional groups on citrulline (Fig. 2). This approach indicates that a majority of protein deimination occurs in the granulocyte nucleus. The direct relation between histone deimination and NETosis was confirmed by Wang and colleagues who showed PAD4-mediated histone PTM in cells undergoing NET release in response to calcium ionophore or TNF [40]. Later, examination of PAD4-deficient mice revealed that PAD4 activity is required for NETosis [41]. Therefore, evidence of deiminated histones has become synonymous with an inflammatory process [42–45]. Once conditions for physiologically induced histone deimination were identified, it became important to test whether deiminated histones serve as preferential substrates for disease-associated autoantibodies.

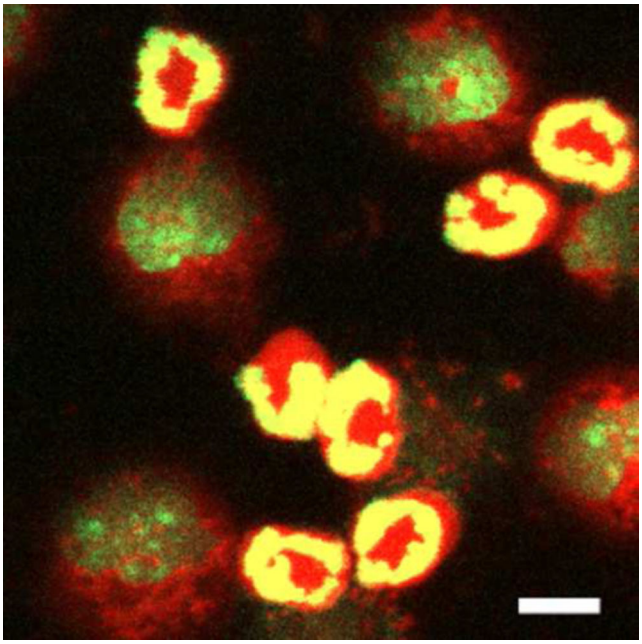

Fig. 2 Chemical detection of citrulline in mouse neutrophils. Mouse neutrophils were elicited by thioglycollate injection into the peritoneal cavity of C57BL/6 mice and collected by lavage. Citrulline residues were detected by phenylglyoxal-rhodamine, a stereospecific dye that reacts with citrulline functional groups at low pH (the details of this procedure will be published elsewhere, Neeli and Radic, in preparation). Neutrophils are easily identified by the shape of their polymorphic nucleus. In addition, there are elicited monocytes in this cytospin preparation. Citrulline residues (*red*) were observed in the nucleus (DNA is stained *green*) and cytoplasm of neutrophils and, to a lesser extent, in the cytoplasm of monocytes. The overlap between the two colors yields *yellow*. The *bar* indicates 10 μm

Autoantibodies to Deiminated Histones

Numerous observations had indicated that activated neutrophils present a more suitable target for certain types of autoantibodies [46]. However, Dwivedi et al. systematically tested the idea that deiminated histones are the preferred antigens of human autoantibodies [47]. First, the authors compared binding of systemic lupus erythematosus (SLE), RA, and Felty's syndrome patients' sera to unstimulated versus LPS-treated neutrophils by confocal microscopy and determined that activated neutrophils and their NETs react more avidly with patient IgG. Second, Dwivedi et al. prepared deiminated histones by incubating purified histones with recombinant PAD4 and observed preferential binding to deiminated histones in ELISA and Western blots. Subsets of SLE and RA sera and essentially all Felty's syndrome sera showed preferential binding. Felty's syndrome is a rare but severe variant of RA. Third, these authors also showed that patients' sera contained substances leading to increased levels of spontaneous NETosis. Thus, a potential vicious cycle of NETosis induction and autoantibodies to NET-associated antigens was discovered [33]. Pratesi and colleagues extended the work by Dwivedi et al. by using citrullinated peptides derived from histone H4 and showing

that the peptides exhibit equal or better discrimination between RA and control sera than citrullinated peptides derived from filaggrin do [48].

Fundamental insights into the relation between NETosis and autoimmunity were derived in a notable series of papers published in 2011. Lupus neutrophils were shown to often include a population of low density granulocytes that exhibit an increased tendency for NETosis [49]. In vivo, these cells potentially account for the observed NET DNA in affected kidneys and skin, along with the increased abundance of NET components in blood that may act as lupus autoantigens [49]. The consequences of an increased NET release in vivo may lead to elevated levels of pro-inflammatory cytokines such as interferon-α (IFN-α) and interleukin-17. The relation between NETs and the production of IFN-α was made explicit by showing that NET components consisting of DNA and the cationic defensin LL37 activate plasmacytoid dendritic cells via toll-like receptor 9 (TLR9) to secrete IFN-α [50]. Additional experiments suggested that SLE patients' neutrophils are primed by IFN-α to release NETs in response to autoantibody-ribonucleoprotein complexes [51]. These experiments cast previously known roles of TLR9 and type I IFN in a new light.

An activated neutrophil subset was also characterized in patients with RA. Studies by Khandpur et al. [52] identified NETting neutrophils in synovial tissues of RA patients and showed that neutrophils are stimulated to undergo NETosis by incubation with ACPA reacting with vimentin. Such NETs had a distinct composition of NET components, as demonstrated by RA NET purification followed by mass spectrometry [52]. In conjunction, these studies strengthened the argument that neutrophils play an important role in the pathogenesis of SLE and RA. Additional evidence supporting the idea that neutrophil activation is responsible for introducing modifications into autoantigens and that these PTM, in turn, stimulate autoantibody production is provided by the prevalence of RA autoantibodies to oxidatively modified autoantigens [53]. However, studies in a mouse model of lupus could not confirm the contribution of NETs to the development of autoimmunity, as breeding to mice incapable of NET release failed to ameliorate the typical pathology or autoantibody production [54]. It is unclear whether differences in Fc receptors between mice and humans may account for the observed differences in experimental outcomes [55]. Additional parallels between PAD deimination and human pathology exist and promise exciting applications of PAD inhibitors in the treatment of cardiovascular diseases [45] and cancer [56].

Histone Deimination and Chromatin Structure

Experiments with autoantibodies to deiminated histones show that studies in autoimmunity have the ability to illuminate basic principles in molecular biology. H1 linker histones are a family of seven isoforms of extranucleosomal histones that occupy the DNA between adjacent nucleosomes [57]. As such, linker histones are in an ideal position to regulate chromatin structure and gene expression [58]. Because it was not known whether H1 histones are deiminated, conditions that strongly induce PAD4 were used to stimulate neutrophils and linker histones were purified based on their unique solubility in 5 % perchloric acid [59]. Mass spectrometry revealed that

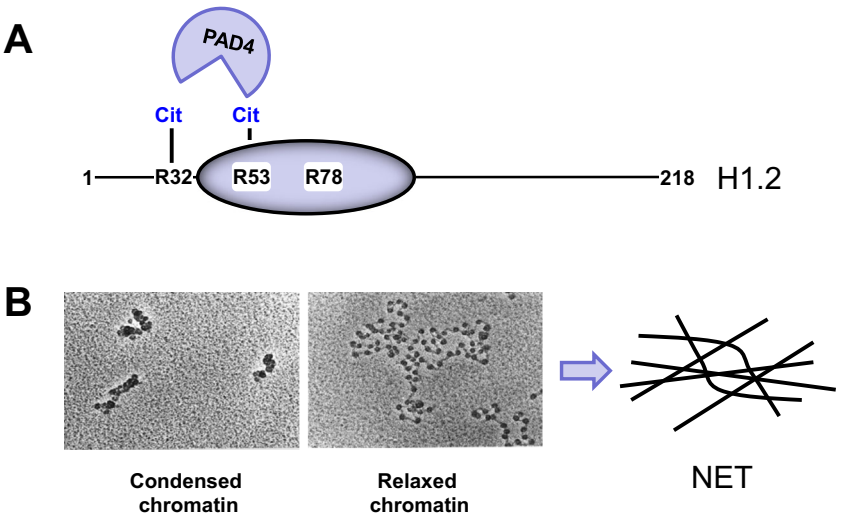

Fig. 3 Histone H1, an important element of chromatin dynamics. **a**:PAD4 converts two of the three arginine residues in H1.2 to citrulline residues in stimulated neutrophils. Whereas Arg32 is located in the unstructured N-terminal tail of H1.2, Arg53 is contained within the globular domain of the protein. The residue Arg 78, also present within the globular domain of H1.2, is not citrullinated. **b** Histone H1 plays a key function in chromatin folding and compaction. H1-depleted chromatin appears relaxed in comparison to native (H1-containing) chromatin fibers. Citrullination of H1 may contribute to its detachment from the chromatin and the subsequent unfolding of chromatin that is required for the relaxed NET chromatin release

only two of the three arginine residues in H1.2 are converted to citrulline residues in neutrophils (Fig. 3), a conclusion that was confirmed in vitro by incubation with the recombinant PAD4 [59]. Peptides containing either of these two citrulline residues were used to determine which citrullinated peptide is the preferred substrate of autoantibodies from SLE or Sjögren's syndrome patients. Even though only about 6 % of SLE sera contained autoantibodies to deiminated H1 histones, the most prevalent binding was to the citrulline residue at position 53 (Fig. 3). This residue is located within the most conserved portion of the H1 helix-turn-helix domain [59]. Residue 53 plays a key role in regulating chromatin structure, a conclusion consistent with recent observations in pluripotent stem cells. Deimination of H1 linker histone by PAD4 is essential for the reprogramming of gene expression that is required during differentiation of pluripotent stem cells into separate cell lineages [60]. Autoantibodies to deiminated H1 linker histones thus identify a crucial switch in chromatin structure that is essential for stem cell gene reprogramming.

Deimination of core histones similarly has broader implications for gene expression. Now, in the classic experiments on histone deimination, Cuthbert et al. [61] and Wang et al. [62] identified hormone responsive genes whose promoters exhibit deiminated histones. Subsequent studies provided additional examples of gene regulation by histone deimination and linked PAD4 to diverse biological processes ranging from mammalian development to tumorigenesis [63–67]. Because PAD4 autocitrullinates and thereby alters autoantibody binding [68], it will be important to determine whether and to what extent autoantibodies that arise is systemic autoimmune disorders affect the function of PAD4 and its specific chromatin substrates.

Summary and Future Perspectives

From nearly a decade of research into autoantibodies to deiminated histones, it stands established that inflammatory conditions lead to a neutrophil cell death that is both antimicrobial and prone to induce autoantibodies to chromatin autoantigens. Autoantibodies to deiminated histones arise in distinct autoimmune disorders and thus provide arguments for the important role of neutrophils in the initial stimulation of the adaptive immune system that leads to autoimmunity. Clearly, it is imperative to pursue studies on deimination and its regulation during NETosis. Early successes of therapies for autoimmune disorders that are based on inhibition of PAD4 suggest that inhibitors of PAD4 will find broad applications in rheumatology clinics. Studies in animal models of autoimmunity have shown significant improvement in disease presentation upon administration of PAD4 inhibitors [3]. The remarkable list of clinical conditions that were improved by PAD4 inhibition includes experimental arthritis, lupus, MS-like disease, and colitis [69–72]. It is reasonable to expect that future efforts to understand and regulate PAD4 will continue to yield real benefits for patients suffering from diverse autoimmune disorders.

Acknowledgments Research in the SM's laboratory is financially supported by the French Centre National de la Recherche Scientifique, Région Alsace, Laboratory of Excellence Medalis (ANR-10-LABX-0034), EquipEx program I2MC (ANR-11-EQPX-022), Initiative of Excellence (IdEx), Strasbourg University, and ImmuPharma France. Research in the MR's laboratory is financially supported by The Lupus Research Institute of New York and the ORR Fund of Memphis, TN. We gratefully acknowledge the kind gift of the anti-PAD4 monoclonal antibody from Dr. Katsuhiko Nakashima of the National Cancer Center Institute of Japan. The authors thank Dr. Gilbert de Murcia, CNRS, who took the original images shown in Fig. 3b. We are grateful to Dr. Indira Neeli and Dr. Teruki Hagiwara for their helpful comments that improved this manuscript.

References

1. Rogers GE, Simmonds DH (1958) Content of citrulline and other amino-acids in a protein of hair follicles. Nature 182:186–187
2. Vossenaar ER, van Zendman AJ, Venrooij WJ, Pruijn GJ (2003) PAD, a growing family of citrullinating enzymes: genes, features and involvement in disease. Bioessays 25:1106–1118
3. Jones JE, Causey CP, Knuckley B, Slack-Noyes JL, Thompson PR (2009) Protein arginine deiminase 4 (PAD4): current understanding and future therapeutic potential. Curr Opin Drug Discov Devel 12: 616–627
4. Mallya RK, Young BJ, Pepys MB, Hamblin TJ, Mace BE, Hamilton EB (1983) Anti-keratin antibodies in rheumatoid arthritis: frequency and correlation with other features of the disease. Clin Exp Immunol 51:17–20
5. Jones JC, Arnn J, Staehelin LA, Goldman RD (1984) Human autoantibodies against desmosomes: possible causative factors in pemphigus. Proc Natl Acad Sci U S A 81:2781–2785
6. Simon M, Girbal E, Sebbag M, Gomes-Daudrix V, Vincent C, Salama G, Serre G (1993) The cytokeratin filament-aggregating protein filaggrin is the target of the so-called "antikeratin antibodies," autoantibodies specific for rheumatoid arthritis. J Clin Invest 92: 1387–1393
7. de Schellekens GA, van den Jong BA, van de Hoogen FH, Putte LB, van Venrooij WJ (1998) Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. J Clin Invest 101:273–281
8. van Venrooij WJ, van Beers JJ, Pruijn GJ (2011) Anti-CCP antibodies: the past, the present and the future. Nat Rev Rheumatol 7:391–398
9. Iobagiu C, Magyar A, Nogueira L, Cornillet M, Sebbag M, Arnaud J, Hudecz F, Serre G (2011) The antigen specificity of the rheumatoid arthritis-associated ACPA directed to citrullinated fibrin is very closely restricted. J Autoimmun 37:263–272
10. Gomara MJ, Haro I (2013) Citrullinated peptides in the diagnosis of rheumatoid arthritis. Curr Top Med Chem 13:743–751
11. Suwannalai P, Britsemmer K, Knevel R, Scherer HU, van der Levarht EW, van Helm-van Mil AH, Schaardenburg D, Huizinga TW, Toes RE, Trouw LA (2014) Low-avidity anticitrullinated protein antibodies (ACPA) are associated with a higher rate of joint destruction in rheumatoid arthritis. Ann Rheum Dis 73:270–276
12. Cornillet M, Sebbag M, Verrouil E, Magyar A, Babos F, Ruyssen-Witrand A, Hudecz F, Cantagrel A, Serre G, Nogueira L (2014) The fibrin-derived citrullinated peptide beta60-74Cit60,72,74 bears the major ACPA epitope recognised by the rheumatoid arthritis-specific

anticitrullinated fibrinogen autoantibodies and anti-CCP2 antibodies. Ann Rheum Dis 73:1246–1252
13. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, Birnbaum NS, Burmester GR, Bykerk VP, Cohen MD, Combe B, Costenbader KH, Dougados M, Emery P, Ferraccioli G, Hazes JM, Hobbs K, Huizinga TW, Kavanaugh A, Kay J, Kvien TK, Laing T, Mease P, Menard HA, Moreland LW, Naden RL, Pincus T, Smolen JS, Stanislawska-Biernat E, Symmons D, Tak PP, Upchurch KS, Vencovsky J, Wolfe F, Hawker G (2010) 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum 62:2569–2581
14. Martin R, Whitaker JN, Rhame L, Goodin RR, McFarland HF (1994) Citrulline-containing myelin basic protein is recognized by T-cell lines derived from multiple sclerosis patients and healthy individuals. Neurology 44:123–129
15. Zhou SR, Whitaker JN, Wood DD, Moscarello MA (1993) Immunological analysis of the amino terminal and the C8 isomer of human myelin basic protein. J Neuroimmunol 46:91–96
16. Haag S, Schneider N, Mason DE, Tuncel J, Andersson IE, Peters EC, Burkhardt H, Holmdahl R (2014) Identification of new citrulline-specific autoantibodies, which bind to human arthritic cartilage, by mass spectrometric analysis of citrullinated type II collagen. Arthritis Rheumatol 66:1440–1449
17. Ogden CA, deCathelineau A, Hoffmann PR, Bratton D, Ghebrehiwet B, Fadok VA, Henson PM (2001) C1q and mannose binding lectin engagement of cell surface calreticulin and CD91 initiates macropinocytosis and uptake of apoptotic cells. J Exp Med 194: 781–795
18. Ling S, Cline EN, Haug TS, Fox DA, Holoshitz J (2013) Citrullinated calreticulin potentiates rheumatoid arthritis shared epitope signaling. Arthritis Rheum 65:618–626
19. Loos T, Mortier A, Gouwy M, Ronsse I, Put W, Van Lenaerts JP, Damme J, Proost P (2008) Citrullination of CXCL10 and CXCL11 by peptidylarginine deiminase: a naturally occurring posttranslational modification of chemokines and new dimension of immunoregulation. Blood 112:2648–2656
20. Proost P, Loos T, Mortier A, Schutyser E, Gouwy M, Noppen S, Dillen C, Ronsse I, Conings R, Struyf S, Opdenakker G, Maudgal PC, Van Damme J (2008) Citrullination of CXCL8 by peptidylarginine deiminase alters receptor usage, prevents proteolysis, and dampens tissue inflammation. J Exp Med 205:2085–2097
21. Moelants EA, Mortier A, Grauwen K, Ronsse IV, Damme J, Proost P (2013) Citrullination of TNF-alpha by peptidylarginine deiminases reduces its capacity to stimulate the production of inflammatory chemokines. Cytokine 61:161–167
22. Bradford CM, Ramos I, Cross AK, Haddock G, McQuaid S, Nicholas AP, Woodroofe MN (2014) Localisation of citrullinated proteins in normal appearing white matter and lesions in the central nervous system in multiple sclerosis. J Neuroimmunol 273:85–95
23. Tarcsa E, Marekov LN, Mei G, Melino G, Lee SC, Steinert PM (1996) Protein unfolding by peptidylarginine deiminase. Substrate specificity and structural relationships of the natural substrates trichohyalin and filaggrin. J Biol Chem 271:30709–30716
24. Shelef MA, Bennin DA, Mosher DF, Huttenlocher A (2012) Citrullination of fibronectin modulates synovial fibroblast behavior. Arthritis Res Ther 14:R240
25. Van Steendam K, Tilleman KD, Ceuleneer MD, Keyser F, Elewaut D, Deforce D (2010) Citrullinated vimentin as an important antigen in immune complexes from synovial fluid of rheumatoid arthritis patients with antibodies against citrullinated proteins. Arthritis Res Ther 12:R132
26. U KP, Subramanian V, Nicholas AP, Thompson PR, Ferretti P (2014) Modulation of calcium-induced cell death in human neural stem cells by the novel peptidylarginine deiminase-AIF pathway. Biochim Biophys Acta 1843:1162–1171
27. Matsuo K, Xiang Y, Nakamura H, Masuko K, Yudoh K, Noyori K, Nishioka K, Saito T, Kato T (2006) Identification of novel citrullinated autoantigens of synovium in rheumatoid arthritis using a proteomic approach. Arthritis Res Ther 8:R175
28. Shoda H, Fujio K, Shibuya M, Okamura T, Sumitomo S, Okamoto A, Sawada T, Yamamoto K (2011) Detection of autoantibodies to citrullinated BiP in rheumatoid arthritis patients and pro-inflammatory role of citrullinated BiP in collagen-induced arthritis. Arthritis Res Ther 13:R191
29. Harlow L, Rosas IO, Gochuico BR, Mikuls TR, Dellaripa PF, Oddis CV, Ascherman DP (2013) Identification of citrullinated hsp90 isoforms as novel autoantigens in rheumatoid arthritis-associated interstitial lung disease. Arthritis Rheum 65:869–879
30. Wegner N, Wait R, Sroka A, Eick S, Nguyen KA, Lundberg K, Kinloch A, Culshaw S, Potempa J, Venables PJ (2010) Peptidylarginine deiminase from Porphyromonas gingivalis citrullinates human fibrinogen and alpha-enolase: implications for autoimmunity in rheumatoid arthritis. Arthritis Rheum 62:2662–2672
31. Amara K, Steen J, Murray F, Morbach H, Fernandez-Rodriguez BM, Joshua V, Engstrom M, Snir O, Israelsson L, Catrina AI, Wardemann H, Corti D, Meffre E, Klareskog L, Malmstrom V (2013) Monoclonal IgG antibodies generated from joint-derived B cells of RA patients have a strong bias toward citrullinated autoantigen recognition. J Exp Med 210:445–455
32. Brink M, Hansson M, Mathsson L, Jakobsson PJ, Holmdahl R, Hallmans G, Stenlund H, Ronnelid J, Klareskog L, Rantapaa-Dahlqvist S (2013) Multiplex analyses of antibodies against citrullinated peptides in individuals prior to development of rheumatoid arthritis. Arthritis Rheum 65:899–910
33. Dwivedi N, Radic M (2014) Citrullination of autoantigens implicates NETosis in the induction of autoimmunity. Ann Rheum Dis 73:483–491
34. Hagiwara T, Nakashima K, Hirano H, Senshu T, Yamada M (2002) Deimination of arginine residues in nucleophosmin/B23 and histones in HL-60 granulocytes. Biochem Biophys Res Commun 290:979–983
35. Neeli I, Khan SN, Radic M (2008) Histone deimination as a response to inflammatory stimuli in neutrophils. J Immunol 180:1895–1902
36. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A (2004) Neutrophil extracellular traps kill bacteria. Science 303:1532–1535
37. Brinkmann V, Zychlinsky A (2012) Neutrophil extracellular traps: is immunity the second function of chromatin? J Cell Biol 198:773–783
38. Kaplan MJ, Radic M (2012) Neutrophil extracellular traps: double-edged swords of innate immunity. J Immunol 189:2689–2695
39. Fuchs TA, Abed U, Goosmann C, Hurwitz R, Schulze I, Wahn V, Weinrauch Y, Brinkmann V, Zychlinsky A (2007) Novel cell death program leads to neutrophil extracellular traps. J Cell Biol 176:231–241
40. Wang Y, Li M, Stadler S, Correll S, Li P, Wang D, Hayama R, Leonelli L, Han H, Grigoryev SA, Allis CD, Coonrod SA (2009) Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation. J Cell Biol 184:205–213
41. Li P, Li M, Lindberg MR, Kennett MJ, Xiong N, Wang Y (2010) PAD4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps. J Exp Med 207:1853–1862
42. Munks MW, McKee AS, Macleod MK, Powell RL, Degen JL, Reisdorph NA, Kappler JW, Marrack P (2010) Aluminum adjuvants elicit fibrin-dependent extracellular traps in vivo. Blood 116:5191–5199
43. Li Y, Liu B, Fukudome EY, Lu J, Chong W, Jin G, Liu Z, Velmahos GC, Demoya M, King DR, Alam HB (2011) Identification of citrullinated histone H3 as a potential serum protein biomarker in a lethal model of lipopolysaccharide-induced shock. Surgery 150:442–451

44. Borissoff JI, Joosen IA, Versteylen MO, Brill A, Fuchs TA, Savchenko AS, Gallant M, Martinod KT, Cate H, Hofstra L, Crijns HJ, Wagner DD, Kietselaer BL (2013) Elevated levels of circulating DNA and chromatin are independently associated with severe coronary atherosclerosis and a prothrombotic state. Arterioscler Thromb Vasc Biol 33:2032–2040
45. Martinod K, Demers M, Fuchs TA, Wong SL, Brill A, Gallant M, Hu J, Wang Y, Wagner DD (2013) Neutrophil histone modification by peptidylarginine deiminase 4 is critical for deep vein thrombosis in mice. Proc Natl Acad Sci U S A 110:8674–8679
46. van Rossum AP, Limburg PC, Kallenberg CG (2005) Activation, apoptosis, and clearance of neutrophils in Wegener's granulomatosis. Ann N Y Acad Sci 1051:1–11
47. Dwivedi N, Upadhyay J, Neeli I, Khan S, Pattanaik D, Myers L, Kirou KA, Hellmich B, Knuckley B, Thompson PR, Crow MK, Mikuls TR, Csernok E, Radic M (2012) Felty's syndrome autoantibodies bind to deiminated histones and neutrophil extracellular chromatin traps. Arthritis Rheum 64:982–992
48. Pratesi F, Dioni I, Tommasi C, Alcaro MC, Paolini I, Barbetti F, Boscaro F, Panza F, Puxeddu I, Rovero P, Migliorini P (2013) Antibodies from patients with rheumatoid arthritis target citrullinated histone 4 contained in neutrophils extracellular traps. Ann Rheum Dis 73:1414–1422
49. Villanueva E, Yalavarthi S, Berthier CC, Hodgin JB, Khandpur R, Lin AM, Rubin CJ, Zhao W, Olsen SH, Klinker M, Shealy D, Denny MF, Plumas J, Chaperot L, Kretzler M, Bruce AT, Kaplan MJ (2011) Netting neutrophils induce endothelial damage, infiltrate tissues, and expose immunostimulatory molecules in systemic lupus erythematosus. J Immunol 187:538–552
50. Lande R, Ganguly D, Facchinetti V, Frasca L, Conrad C, Gregorio J, Meller S, Chamilos G, Sebasigari R, Riccieri V, Bassett R, Amuro H, Fukuhara S, Ito T, Liu YJ, Gilliet M (2011) Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. Sci Transl Med 3:73ra19
51. Garcia-Romo GS, Caielli S, Vega B, Connolly J, Allantaz F, Xu Z, Punaro M, Baisch J, Guiducci C, Coffman RL, Barrat FJ, Banchereau J, Pascual V (2011) Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. Sci Transl Med 3:73ra20
52. Khandpur R, Carmona-Rivera C, Vivekanandan-Giri A, Gizinski A, Yalavarthi S, Knight JS, Friday S, Li S, Patel RM, Subramanian V, Thompson P, Chen P, Fox DA, Pennathur S, Kaplan MJ (2013) NETs are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis. Sci Transl Med 5:178ra140
53. Strollo R, Ponchel F, Malmstrom V, Rizzo P, Bombardieri M, Wenham CY, Landy R, Perret D, Watt F, Corrigall VM, Winyard PG, Pozzilli P, Conaghan PG, Panayi GS, Klareskog L, Emery P, Nissim A (2013) Autoantibodies to posttranslationally modified type II collagen as potential biomarkers for rheumatoid arthritis. Arthritis Rheum 65:1702–1712
54. Campbell AM, Kashgarian M, Shlomchik MJ (2012) NADPH oxidase inhibits the pathogenesis of systemic lupus erythematosus. Sci Transl Med 4:157ra141
55. Tsuboi N, Ernandez T, Li X, Nishi H, Cullere X, Mekala D, Hazen M, Kohl J, Lee DM, Mayadas TN (2011) Regulation of human neutrophil Fcgamma receptor IIa by C5a receptor promotes inflammatory arthritis in mice. Arthritis Rheum 63:467–478
56. McElwee JL, Mohanan S, Horibata S, Sams KL, Anguish LJ, McLean D, Cvitas I, Wakshlag JJ, and Coonrod S (2014) PAD2 overexpression in transgenic mice promotes spontaneous skin neoplasia. Cancer Res. doi:10.1158/0008-5472.CAN-14-0749
57. Happel N, Doenecke D (2009) Histone H1 and its isoforms: contribution to chromatin structure and function. Gene 431:1–12
58. Harshman SW, Young NL, Parthun MR, Freitas MA (2013) H1 histones: current perspectives and challenges. Nucleic Acids Res 41:9593–9609
59. Dwivedi N, Neeli I, Schall N, Wan H, Desiderio DM, Csernok E, Thompson PR, Dali H, Briand JP, Muller S, Radic M (2014) Deimination of linker histones links neutrophil extracellular trap release with autoantibodies in systemic autoimmunity. FASEB J 28: 2840–2851
60. Christophorou MA, Castelo-Branco G, Halley-Stott RP, Oliveira CS, Loos R, Radzisheuskaya A, Mowen KA, Bertone P, Silva JC, Zernicka-Goetz M, Nielsen ML, Gurdon JB, Kouzarides T (2014) Citrullination regulates pluripotency and histone H1 binding to chromatin. Nature 507:104–108
61. Cuthbert GL, Daujat S, Snowden AW, Erdjument-Bromage H, Hagiwara T, Yamada M, Schneider R, Gregory PD, Tempst P, Bannister AJ, Kouzarides T (2004) Histone deimination antagonizes arginine methylation. Cell 118:545–553
62. Wang Y, Wysocka J, Sayegh J, Lee YH, Perlin JR, Leonelli L, Sonbuchner LS, McDonald CH, Cook RG, Dou Y, Roeder RG, Clarke S, Stallcup MR, Allis CD, Coonrod SA (2004) Human PAD4 regulates histone arginine methylation levels via demethylimination. Science 306:279–283
63. Zhang X, Gamble MJ, Stadler S, Cherrington BD, Causey CP, Thompson PR, Roberson MS, Kraus WL, Coonrod SA (2011) Genome-wide analysis reveals PADI4 cooperates with Elk-1 to activate c-Fos expression in breast cancer cells. PLoS Genet 7:e1002112
64. Kan R, Jin M, Subramanian V, Causey CP, Thompson PR, Coonrod SA (2012) Potential role for PADI-mediated histone citrullination in preimplantation development. BMC Dev Biol 12:19
65. Stadler SC, Vincent CT, Fedorov VD, Patsialou A, Cherrington BD, Wakshlag JJ, Mohanan S, Zee BM, Zhang X, Garcia BA, Condeelis JS, Brown AM, Coonrod SA, Allis CD (2013) Dysregulation of PAD4-mediated citrullination of nuclear GSK3beta activates TGF-beta signaling and induces epithelial-to-mesenchymal transition in breast cancer cells. Proc Natl Acad Sci U S A 110:11851–11856
66. Tanikawa C, Espinosa M, Suzuki A, Masuda K, Yamamoto K, Tsuchiya E, Ueda K, Daigo Y, Nakamura Y, Matsuda K (2012) Regulation of histone modification and chromatin structure by the p53-PADI4 pathway. Nat Commun 3:676
67. Wang Y, Li P, Wang S, Hu J, Chen XA, Wu J, Fisher M, Oshaben K, Zhao N, Gu Y, Wang D, Chen G (2012) Anticancer peptidylarginine deiminase (PAD) inhibitors regulate the autophagy flux and the mammalian target of rapamycin complex 1 activity. J Biol Chem 287:25941–25953
68. Andrade F, Darrah E, Gucek M, Cole RN, Rosen A, Zhu X (2010) Autocitrullination of human peptidyl arginine deiminase type 4 regulates protein citrullination during cell activation. Arthritis Rheum 62:1630–1640
69. Willis VC, Gizinski AM, Banda NK, Causey CP, Knuckley B, Cordova KN, Luo Y, Levitt B, Glogowska M, Chandra P, Kulik L, Robinson WH, Arend WP, Thompson PR, Holers VM (2011) N-alpha-benzoyl-N5-(2-chloro-1-iminoethyl)-L-ornithine amide, a protein arginine deiminase inhibitor, reduces the severity of murine collagen-induced arthritis. J Immunol 186:4396–4404
70. Knight JS, Zhao W, Luo W, Subramanian V, O'Dell AA, Yalavarthi S, Hodgin JB, Eitzman DT, Thompson PR, Kaplan MJ (2013) Peptidylarginine deiminase inhibition is immunomodulatory and vasculoprotective in murine lupus. J Clin Invest 123:2981–2993
71. Wei L, Wasilewski E, Chakka SK, Bello AM, Moscarello MA, Kotra LP (2013) Novel inhibitors of protein arginine deiminase with potential activity in multiple sclerosis animal model. J Med Chem 56: 1715–1722
72. Chumanevich AA, Causey CP, Knuckley BA, Jones JE, Poudyal D, Chumanevich AP, Davis T, Matesic LE, Thompson PR, Hofseth LJ (2011) Suppression of colitis in mice by Cl-amidine: a novel peptidylarginine deiminase inhibitor. Am J Physiol Gastrointest Liver Physiol 300:G929–938
73. Van Moelants EA, Damme J, Proost P (2011) Detection and quantification of citrullinated chemokines. PLoS One 6:e28976