

## EDITORIAL

# Inhibiting citrullination in rheumatoid arthritis: taking fuel from the fire

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See related research by Makrygiannakis *et al.*, <http://arthritis-research.com/content/14/1/R20>

### Abstract

Citrullination is a post-translational modification catalysed by peptidylarginine deiminase and is a common feature of inflammation. The presence of anti-citrullinated protein/peptide antibodies (ACPA), however, is unique to rheumatoid arthritis. Several lines of evidence suggest that ACPA are important in the pathogenesis of rheumatoid arthritis. A popular hypothesis for this pathogenesis is a two-hit model. The first hit gives rise to ACPA, and the second hit, an unrelated episode of synovial inflammation accompanied by citrullination, is perpetuated by the pre-existing antibodies. This model suggests that reducing citrullination might ameliorate disease. Recent findings indicate that citrullination closely correlates with inflammation, and that glucocorticoids decrease peptidylarginine deiminase expression independent of their other anti-inflammatory effects.

In this issue, Makrygiannakis and colleagues study the effect on synovial citrullination of treatment with two commonly used drugs in the treatment of rheumatoid arthritis (RA) [1]. They found by immunohistochemistry that intracellular citrullination, as determined by F95 antibody staining, as well as peptidylarginine deiminase (PAD) expression were correlated with measures of synovial inflammation. Intra-articular injection of glucocorticoid, but not oral methotrexate, was associated with a reduction in synovial inflammation, intracellular citrullination, and PAD expression. Based on cultures of synovial fluid mononuclear cells and synovial explants, they also make the interesting proposal that glucocorticoids may suppress citrullination independent of inflammation

by inhibiting PAD enzyme expression. There are a number of caveats to this proposition, including the specificity of anti-PAD antibodies. In our laboratory we have found a number of these antibodies to be cross-reactive with other proteins, which could confound immunohistochemistry findings unless specificity is confirmed or the results are corroborated by other techniques. Nevertheless, given the dearth of information on the regulation of citrullination in RA, their paper is welcome.

The presence of anti-citrullinated protein/peptide antibodies (ACPA) defines a major subset of RA that is associated with distinctive genetic and environmental risk factors and with a more severe clinical phenotype [2]. Frequently cited evidence to support the importance of ACPA in pathogenesis includes their appearance years before clinical diagnosis, their production within the joint, the ability of ACPA immune complexes to activate macrophages, and some animal model data [2]. The actual role of ACPA in pathogenesis is still a matter for investigation. A widely-held hypothesis for this pathogenesis comprises two hits [2]. The first hit follows accelerated citrullination of proteins in an extra-articular site – due to smoking or infection, for example – which in the context of a permissive HLA type gives rise to ACPA. The second hit, which may occur years later, would be an unrelated episode of otherwise self-limiting synovial inflammation. Since citrullination of proteins is a feature of inflammatory tissue, the presence of pre-existing ACPA would exacerbate and perpetuate the synovitis. If this was to be the case, one might predict that inhibiting citrullination would ameliorate disease – the findings of Makrygiannakis and colleagues suggest this may be a previously unappreciated mechanism of action of glucocorticoids [1].

Such a hypothesis argues for investigation of specific PAD inhibitors. The chemotherapeutic drug paclitaxel inhibits PAD enzymes and is efficacious in a rat collagen-induced arthritis model [3]. This agent has other notable effects relevant to RA, however, including inhibition of microtubule formation and angiogenesis. More recently, Willis and colleagues reported that the pan-PAD

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irreversible inhibitor Cl-amidine partially inhibited arthritis in a mouse collagen-induced arthritis model [4]. They observed a reduction in antibody levels to native, but not bovine, collagen, and to a limited number of other candidate autoantigens that they studied by microarray. The only reduction in ACPA reactivity was to filaggrin. Interestingly, despite a reduction in clinical and histological arthritis scores, synovial infiltration by immune cells was unaffected. Given that ACPA autoimmunity is unlikely to be of major importance in collagen-induced arthritis, the role of citrullination in this model, the mechanism of action of Cl-amidine, and the relevance of the findings to RA are all unclear.

The safety of PAD inhibition is a major consideration. The physiological role of the different PAD enzymes is incompletely understood but there are proteins in the stratum corneum and myelin sheath that are constitutively deiminated. Citrullination also appears to play a role in apoptosis, formation of neutrophil extracellular traps, altering chemokine function and, in the case of PAD4, regulating DNA transcription [5]. Only PAD2 and PAD4 have been identified in the synovium [6] and most attention in RA has focused on PAD4, which has a more limited tissue distribution being mainly expressed in leucocytes. *PADI4* polymorphisms are associated with RA in East Asian populations [2], and autocitrullinating PAD4 is itself an autoantigen in RA [7]. Whilst PAD2 can be found in the synovium of both osteoarthritis and RA, Foulquier and colleagues could only identify PAD4 in inflammatory synovitis [6]. It is therefore of interest that Makrygiannakis and colleagues found glucocorticoids to reduce PAD4 consistently, but not PAD2 [1].

PAD2 levels, however, are known to increase as monocytes differentiate into macrophages, which make up a large proportion of lining cells in RA synovium [8]. Studies of synovial fluid in our laboratory showed that PAD4 could be found in both osteoarthritis and inflammatory arthritis, whereas PAD2 was limited to inflammatory disease [9]. Notably, Makrygiannakis and colleagues found synovial PAD2 levels to correlate with inflammation rather better than PAD4 [1].

Only a small number of potentially citrullinated proteins are antigenic targets in RA, and evidence is emerging that PAD2 and PAD4 differ in their substrate specificity [10]. Further studies of these enzymes are needed to better understand their regulation, their relative contribution to citrullination in RA and, in particular, their substrate specificity, to guide therapeutic development. Vossenaar and van Venrooij have described citrullinated proteins as

'sparks that may ignite the fire of RA' [11]. The findings of Makrygiannakis and colleagues suggest that PAD enzymes may also provide the fuel that keeps the fire burning, and that their inhibition may be a key target for novel therapy.

#### Abbreviations

ACPA, anti-citrullinated protein/peptide antibodies; PAD, peptidylarginine deiminase; RA, rheumatoid arthritis.

#### Competing interests

The authors declare that they have no competing interests.

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