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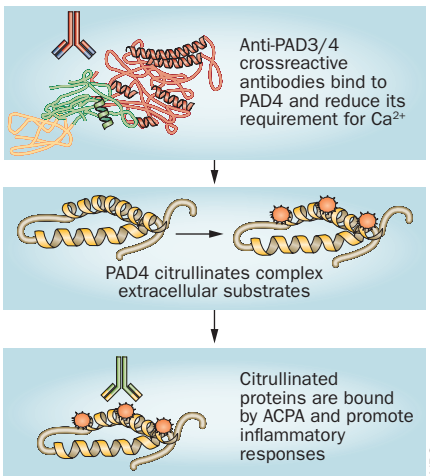
RHEUMATOID ARTHRITIS

PAD-ing out the ACPA response in RA

Autoantibodies targeting both protein–arginine deiminase type-3 (PAD3) and PAD4 augment the enzymatic activity of PAD4, which might underlie the association of these crossreactive autoantibodies with erosive rheumatoid arthritis (RA), according to a study just published in *Science Translational Medicine*. The data "could explain how PAD enzymes ... work in the extracellular space, where calcium concentrations are generally too low to allow this enzyme to work optimally," notes Leendert Trouw, of Leiden University Medical Centre, Netherlands, an expert in autoantibodies in RA who was not involved in the study.

PADs catalyse protein citrullination, a process that is important for the generation of autoantigens in RA. PAD4 itself is also known to be targeted by autoantibodies in some patients with RA, and the presence of these antibodies has been linked to increased disease severity. However, until now, the mechanisms underlying this association were unclear.

In their new study, Erika Darrah *et al.* identified a subset of anti-PAD4 antibodies from patients with RA through their crossreactivity with PAD3, and set out to investigate their effect on disease using serum samples and radiographic data from 194 patients in the ESCAPE RA cohort. The anti-PAD3/4 antibodies were detected in 12% of the patients, and in 32% of those with anti-PAD4 antibodies, but not in healthy controls ($n = 36$) or in patients with psoriatic arthritis ($n = 30$). Patients with anti-PAD3/4 antibodies were found to have increased disease severity at baseline, as measured by the Sharp–van der Heijde (SvdH) score, compared with both anti-PAD-negative patients and patients with anti-PAD4 antibodies only. In addition, comparison of the SvdH scores of 150 patients at follow-up (after an average of 39 ± 4 months) showed that patients with these antibodies were also more likely than anti-PAD-negative patients to exhibit radiographic progression. Crucially, adjustments to account for other variables affecting radiographic disease severity (namely age, gender, disease duration, anti-citrullinated protein antibody [ACPA] status, the presence of shared epitope alleles and C-reactive protein levels) weakened but did not abolish these associations.



So, do anti-PAD3/4 antibodies have a pathogenic role in RA? Previous studies have detected soluble PAD2 and PAD4, together with citrullinated autoantigens, in synovial fluid from patients with RA. Thus, it had been suggested that these enzymes could function extracellularly. However, as Darrah explains: "A paradox in the field was the high calcium concentrations required for PAD activity *in vitro*, but their ability to citrullinate proteins *in vivo* with much less calcium present."

Attempting to solve this mystery, the investigators explored whether anti-PAD3/4 antibodies have an impact on the enzymatic function of PAD4, using the canonical PAD4 substrate histone H3. Intriguingly, although the antibodies had little effect on the citrullination of histone H3 at optimal calcium concentrations, they markedly enhanced citrullination at lower, physiological calcium levels. Further experiments to define the molecular basis for this effect suggested that these autoantibodies induce a structural change in PAD4 similar to that caused by calcium binding, which is necessary for the citrullination of macromolecular targets. "The antibody might be hijacking a pathway normally used by other protein partners in the cell to control the sensitivity of PADs to calcium," suggests Darrah, who plans to search for such partners in future experiments.

The findings thus indicate that anti-PAD3/4 antibodies might promote the citrullination of macromolecular substrates in the synovial fluid. Such an increase in the generation of citrullinated autoantigens might augment the generation and binding of ACPA, leading to an enhanced inflammatory response that promotes further joint damage. "Although it was already known that anti-PAD4 antibodies existed in RA, the further identification of a subset of anti-PAD3/4 crossreactive antibodies as a different reactivity from antibodies that target PAD4 only, with clear functional consequences for PAD4, is important and novel," remarks Trouw.

In addition to improving our understanding of the mechanisms of autoantigen generation in RA, does this study have clinical implications? Indeed, according to RA expert Kazuhiko Yamamoto, from the University of Tokyo, Japan: "These findings indicate that a subset of patients with RA who have severe arthritis with erosions could be identified using these autoantibodies." Such a biomarker might help clinicians to target treatments more effectively. Lastly, the work also provides support for new therapeutic avenues: "Citrullination by PAD4 was again suggested to be closely related to RA pathogenesis, and thus PAD inhibitor therapies could be promising," concludes Yamamoto.

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Original article Darrah, E. *et al.* Erosive rheumatoid arthritis is associated with antibodies that activate PAD4 by increasing calcium sensitivity. *Sci. Transl. Med.* **5**, 186ra65 (2013)