PPAD is capable of autocitrullination and able to raise an immune response in RA.

Materials and methods PPAD was expressed as a GST/His-tagged fusion protein using a bacterial expression system. Site-directed mutagenesis using a PCR based method was used to create a PPAD mutant (mPPAD) with a single residue mutation (cysteine 351 to alanine) and with no enzymatic activity. Citrullination was determined by immunoblotting with an antimodified citrullinated antibody kit (AMC, New York, NY, USA). Mass spectrophotometry was carried out to verify PPAD-GST citrullination. ELISAs using recombinant PPAD and recombinant mPPAD were developed to test sera from patients with periodontitis (PD) (n=44), RA (n=82) and healthy controls (C) (n=80). Reactivity was expressed as arbitrary units per ml (AU/ml) using a standard curve.

Results Sera from patients with RA, PD and healthy controls were tested for IgG antibodies to PPAD and showed that RA sera have a significantly elevated antiPPAD IgG response (mean 182.4 AU/ml) compared to PD (mean 96.4AU/ml; $p<0.01$) and healthy control sera (mean 99.2AU/ml; $p<0.05$). An ELISA with the enzymatically non-active, C351A mutated PPAD (mPPAD), showed that the antibody response in the RA group was no longer significantly elevated compared to the antibody responses in the PD and healthy control sera. Antimodified citrulline immunoblotting demonstrated that recombinant PPAD was citrullinated whereas the mPPAD was not. Citrullination of PPAD was confirmed by mass spectrophotometry which showed that 7 of the 18 arginines in PPAD were citrullinated. All citrullines detected were internal and no c-terminal citrullination was observed.

Conclusions The authors present evidence that PPAD is capable of autocitrullination and its target site is not restricted to c-terminal peptide arginines. The antibody response to autocitrullinated PPAD was significantly elevated in RA patients compared to PD patients and controls. This indicates that PPAD is a potential novel antigen in RA and may form part of the anticitrullinated protein antigen response seen in this disease, thus substantiating a possible link between PD and RA.

2 AUTODEIMINATION OF *PORPHYROMONAS GINGIVALIS* PEPTIDYLARGININE DEIMINASE: A NOVEL ANTIGEN IN RHEUMATOID ARTHRITIS

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Background and objectives *Porphyromonas gingivalis*, a major periodontal pathogen, has been implicated in the pathogenesis of rheumatoid arthritis (RA). It is the only pathogenic prokaryote known to possess a bacterial peptidylarginine deiminase (PPAD), an enzyme that catalyses the post-translational modification of arginine to citrulline. The enzyme is surface expressed and citrullinates c-terminal arginines of human fibrinogen and enolase peptides. Here, the authors show that