5 OUTER MEMBRANE VESICLES CONTAINING AUTOCITRULLINATED PEPTIDYLARGININE DEIMINASE FROM *PORPHYROMONAS GINGIVALIS* PART OF AN INFECTION MODEL IN RA?

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Background Bacteria release outer membrane vesicles (OMV) which do contain proteins, DNA and enzymes like peptidylarginine deiminase (PPAD) from gingivalis to interact with bacteria and the host immune system. PPAD has been implicated in the pathogenesis of rheumatoid arthritis (RA). The authors have examined were PPAD its modified in the immune response which patients and controls do respond to *Porphyromonas gingivalis* OMV (POMV) and PPAD.

Methods PPAD was analysed in POMVs, recombinantly expressed and separated by 2-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (2D SDS-PAGE). PPAD was analysed my mass spectrometry (MS). Modifications and the immune reactivity was tested with sera in ELISA and immunoblotting.

Results The authors have analysed the POMV reactivity and found significant reactions of RA patients and controls to the PPAD and other proteins of the POMV. The authors could show that PPAD in OMVs is cleaved rendering it into an active enzyme. POMVs can interact with histones to protect bacteria from antibacterial response of histones. Moreover the anti H1 histone antibody response of systemic lupus erythematosus (SLE) sera can be blocked by POMVs. H1 histone citrullinated with rabbit PAD was positive with anticitrullinated peptide antibodies (ACPA) positive and negative RA and SLE sera. Interestingly PPAD incubated with H1 histone gave no additional reactivity with RA sera and controls. Out of 69 early RA sera, and 23 periodontitis sera (PD), 10 SLE and 10 healthy controls 100% of the early RA and PD sera, 70% of the SLE and 90% of the healthy control sera were reactive with the recombinant PPAD. This immune reactivity correlated with the positivity to *P gingivalis* lipopolysaccharides ELISA. Specific response to the citrullinated form of PPAD could be detected with affinity purified ACPA antibodies from different citrullinated peptides only with RA sera. The human affinity purified patient ACPA antibodies reacted specifically with the 2D SDS-PAGE separated autocitrullinated form of PPAD.

Autocitrullination of PPAD was detected by MS, with human anti cit-fibrinogen antibodies and the antimodified citrulline kit. PPAD citrullination activity was tested with different peptide sequences containing arginine. Sequence and pH dependent cit-reactions could be detected.

Conclusion PPAD autocitrullination and sequence and pH dependent intramolecular citrullination can be detected using different methods. Anti OMV and PPAD antibody reaction is commonly found in RA, SLE and healthy controls. ACPA specific binding with the autocitrullinated form of PPAD can be only detected in RA sera.