A6.3 IMMUNISATION WITH RECOMBINANT AUTOCITRULLINATED *PORPHYROMONAS GINGIVALIS* PEPTIDYLARGININE DEIMINASE INDUCES AUTOIMMUNITY TO ENOLASE AND ARTHRITIS IN DBA/1 MICE

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Background and Objectives Rheumatoid arthritis (RA) is characterised by the presence of anti-citrullinated peptide antibodies (ACPA) years before disease onset. Increasing molecular and epidemiological evidence has linked periodontitis (PD) to RA. *Porphyromonas gingivalis* is unique amongst periodontal pathogens in possessing a citrullinating enzyme, peptidylarginine deiminase (PPAD) with the potential to generate citrullinated antigens driving the autoimmune response in RA. We have examined the immune response to several peptides/proteins of significance to RA in DBA/1 mice immunised with recombinant PPAD.

Materials and Methods Twelve week old DBA/1 mice were immunised with one of two emulsions: 1) recombinant PPAD in complete Freund's adjuvant (CFA) or 2) an inactive PPAD mutant (C351A) in CFA. Clinical score and paw swelling of mice (indicative of arthritis) recorded for ten days post onset. Antibody responses to PPAD and C351A, and a number of immunodominant ACPA target peptides: anti-citrullinated a-enolase peptide-1 (CEP1), vimentin (cVim), fibrinogen (cFib) and their uncitrullinated forms (REP-1, vim and fib) were examined in mouse serum using Enzyme-linked Immunosorbant assays (ELISAs). The Mann-Whitney U test was used to calculate p-values for differences between the sera groups for each antigen.

Results By day 30 post immunisation, 20% of mice immunised with PPAD had developed arthritis-like swelling in their paws. There was no significant difference between the antibody response to PPAD and the antibody response to C351A in any of the mice tested. There was a significantly raised antibody response ($p < 0.05$) to both CEP1 and REP1 (mean 0.263; OD^{450}) in the mice immunised with PPAD compared to the mice immunised with C351A (CEP1, mean 0.074 (OD^{450}) and REP1 mean 0.150 (OD^{450}). Antibody responses to cFib and Fib were similar in all mice, as were antibody responses to cVim and Vim.

Conclusions The paw swelling and raised immune response to the immunodominant enolase peptide, both citrullinated (CEP1) and uncitrullinated (REP1), in mice immunised with autocitrullinated PPAD shows that PPAD induces arthritis and autoimmunity to enolase. This demonstrates that an active citrullinating PPAD can break tolerance to a major RA autoantigen and provides further molecular evidence linking *P. gingivalis* infection to RA.

A6.4 PERIODONTOPATHOGENS IN RHEUMATOID ARTHRITIS AND PERIODONTAL DISEASE

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Background and Objectives A relationship between rheumatoid arthritis (RA) and periodontitis is suggested. Pathogenesis of periodontitis as one of the most common chronic infectious diseases is thought to be host response on subgingival plaque. Among species known to be associated with severe periodontitis, *Porphyromonas gingivalis* plays an important role. Its virulence is most related to cysteine proteases. Moreover, a peptidylarginine deiminase was described to be able to citrullinate microbial and host proteins. The aim of this study was to characterise a group of RA patients for several variables associated with RA and/or periodontitis in comparison with periodontally healthy and periodontitis subjects without RA. In a first part, clinical data of periodontitis and the load of selected periodontopathic species were analysed.

Methods 51 patients with RA, 27 patients with periodontitis and without RA as well as 16 subjects without periodontitis and RA were recruited. Periodontal disease status was determined by using Periodontal Screening index (PSI). Subgingival plaque was analysed semi-quantitatively by PCR followed by a reverse hybridisation (microIdent, Hain Lifescience) for *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis*, *Prevotella intermedia, Tannerella forsythia and Treponema denticola*. In addition, real-time PCR was used to detect very low loads of *P. gingivalis* (detection level 10 bacteria). For statistical analysis Kruskal-Wallis and Mann-Whitney tests were used.

Results Among the 51 RA patients, 45 were characterised positively for periodontitis. 18 (35%) had a severe periodontitis (PSI 4). Analysing the four subgroups (incl. RA with/without periodontitis) showed differences in *A. actinomycetemcomitans* ($p = 0.043$) and *T. denticola* ($p = 0.028$). *P. gingivalis* was detected in 63% of the RA patients and in 49% of the subjects without RA. In all RA patients and in special without periodontitis, *A. actinomycetemcomitans* was found more often ($p = 0.018$ for all, $p = 0.007$ for subjects without periodontitis). In both RA and non-RA subjects, patients with periodontitis had more *T. denticola* in their plaque ($p = 0.026$; $p = 0.042$).

Conclusions *P. gingivalis* induces immune responses which may be of relevance in RA pathogenesis, but other microbes may also play a role in RA associated periodontitis.

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A6.5 SYNOVIAL LYMPHOID STRUCTURES SUPPORT EPSTEIN-BARR VIRUS PERSISTENCE AND AUTOREACTIVE PLASMA CELL INFECTION IN RHEUMATOID ARTHRITIS

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Objectives Rheumatoid arthritis (RA) is associated with an increased Epstein-Barr virus (EBV) blood DNA load, a robust immune response to EBV and cross-reactive circulating antibodies for viral and self-antigens. However, the role of EBV in RA pathogenesis remains elusive. Here we investigated the relationship between synovial EBV infection, ectopic lymphoid structures (ELS) and immunity to citrullinated self and EBV proteins.

Methods Latent and lytic EBV infection was investigated in 43 RA synovial tissues characterised for presence/absence of ELS and 11 OA samples by RT-PCR, in situ hybridisation and immunohistochemistry/immunofluorescence. Synovial production of anti-citrullinated proteins (ACPA) and anti-citrullinated EBV peptides (VCP1/VCP2) antibodies was investigated in situ or in vivo in the SCID/RA chimeric model.

Results EBV dysregulation was observed exclusively in ELS+ RA, but not OA, synovia as revealed by presence of EBV latent [LMP2A, EBV-encoded small RNA (EBER)] transcripts and EBER+ cells and immunoreactivity for EBV latent (LMP1, LMP2A) and lytic (BFRF1) antigens in ELS-associated B cells and plasma cells, respectively. Importantly, ~20% of synovial plasma cells producing ACPA were